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THE INCORPORATION OF DIRECT WITH PROTECTIVE INSECTICIDES AND FUNGICIDES

II. THE EFFECTS OF SPRAY SUPPLEMENTS ON THE RETENTION AND TENACITY OF PROTECTIVE DEPOSITS

By E. FAJANS and H. MARTIN
Agricultural and Horticultural Research Station, Long Ashton, Bristol

INTRODUCTION.

THE field efficiency of protective spray materials, such as Bordeaux mixture, lead arsenate and lime sulphur, which function through the formation of a deposit which protects the foliage from disease organisms or which limits the depredations of insect pests, is partly determined by the amount of deposit which persists upon the foliage throughout the period of attack. For convenience, the amount of deposit at any given time may be termed the spray residue. It is possible, by the addition of certain types of spray supplement to increase, in a variety of ways, the spray residue, such supplements being known by the general term "stickers". The purpose of the investigations to be described below is the examination of the action of such stickers and, in particular, the effects of wetting agents, added to combined washes to promote the efficiency of the direct insecticide or fungicide, on the spray residue of the protective constituent.

The practical problem is, however, not entirely solved by the investigation of the effect of the supplement upon spray residue for, with most protectants, the spray deposit is not primarily the active insecticide or fungicide. With lead arsenate, for example, the actual poisoning of the insect is probably due to the solution of part of the arsenate within the insect's intestine. The efficiency of the arsenical deposit is therefore conditioned by the relative amount of arsenate which is thus rendered soluble. Similarly, the constituent of the Bordeaux deposit which actively prevents the establishment of fungal infection is generally considered to be a soluble copper compound, derived by various agencies from the spray residue. The field performance of a protective spray material will therefore be dependent not only on factors associated with residue but also on those which determine the ease of formation of the active insecticide or fungicide. This latter property may conveniently be termed the availability of the residue.

The quantity of spray residue will, in turn, be determined by at least two factors, first the actual amount of protectant retained on the foliage at spray application and second the ability of the initial deposit to withstand the various agencies which tend to its removal. The former quantity is termed the initial retention and has been the subject of investigations described in Part I of this series (1). The second factor, the resistance of the spray deposit to weathering, is termed the tenacity* of the deposit. In addition it is probable that qualities of the spray residue such as the uniformity of deposit, or "coverage", will influence protective efficiency. This influence will, however, be exerted through the availability factors, in so far as the surface area of the deposit exposed to the action of availability agents is increased, and through tenacity in so far as uneven distribution will increase the effect of weathering agencies. The plan of the work to be described in the present paper is, accordingly, the laboratory investigation of the influence of supplements upon the initial retention and tenacity of protective spray materials applied in the form of suspensions, followed by an examination of the applicability of the laboratory conclusions to field conditions.

LABORATORY TRIALS.

Experimental Methods.

(1) *Suspensions used.*—In selecting a substance suitable for use as the solid phase of the suspensions, the primary requirements were, (1), a compound the analytical determination of which in small amounts is simple, accurate and rapid, and (2), a compound which could be prepared when required as a dispersion of standard physical properties, in particular, particle size and degree of flocculation. The first requirement would be met by an insoluble copper compound and it was found that a suspension of cuprous iodide of suitable rate of sedimentation could be obtained by precipitation from saturated cupric sulphate solution on adding a slight excess of potassium iodide solution, sulphur dioxide being passed through to avoid the formation of free iodine. The cuprous iodide was washed free from excess iodide and potassium sulphate by sedimentation. After ten washings, the volume was adjusted, the spray supplement being added at this stage, to give a suspension of 1% weight cuprous iodide.

In later work, when the amount of suspension required could be foretold, a commercial red cuprous oxide was used. This preparation, containing

* The authors are indebted to Mr. R. W. Marsh for the suggestion of tenacity to define this quality. The expressions "initial retention" and "tenacity" have been adopted in favour of "initial adhesiveness" and "adherence" recently employed by Magie and Horsfall (3) for these two factors. It was considered that the latter terms suggest mechanical sticking, which does not appear to be involved in the determination of initial retention and which may not always be the mechanism responsible for tenacity. Retention and tenacity are terms which imply no hypothesis of the cause of the phenomena.

97% Cu_2O produced by an electrolytic process, was relatively free from the agglomerates present in the cuprous iodide. The particle size was such that by gentle agitation the spray could be kept in uniform dispersion.

(2) *Surfaces used.*—The artificial surfaces employed were glass coated with either paraffin wax, cellulose acetate or cellulose nitrate, particulars of which are given in Part I of this series. An additional surface to be used was of glass coated with a thermal-hardening glyptal resin. The glass plates were immersed in a 2·5% solution of the resin in Cellosolve (ethylene glycol mono-ethyl ether) and stoved at 120-30° C. for one hour.

(3) *The estimation of Initial Retention.*—Working with simple solutions, Evans and Martin showed that the maximum quantity of spray of wetting properties of the range under examination which could be carried by a vertical surface held at right angles to the direction of the spray is identical with the quantity applied at the point immediately prior to run-off or drain-off. The amount may therefore be determined, using the atomiser and pendulum method of application previously described, by multiplying the volume applied per square inch per exposure by the number of exposures. Before this method could be applied to the estimation of the initial retention of solid particles from a suspension, it was necessary to establish that there is no preferential retention of the solid phase in the system under investigation. For this purpose, paraffin and cellulose acetate surfaces were sprayed with 1% aqueous suspensions of cuprous iodide, the volume of spray applied being regulated by the number of swings of the pendulum and the aperture between the surface and the atomiser. To delimit the area of surface exposed to the spray it was covered by a glass plate with an aperture one inch square. The amount of copper retained upon the square inch of exposed surface was determined by solution in warm dilute

Table I.

Retention of 1% Cuprous Iodide Suspensions.

Paraffin wax surface.			Cellulose acetate surface.		
No. of exposures.	mg. Cu.		No. of exposures.	mg. Cu.	
	Applied.	Retained.		Applied.	Retained.
50	0·49	0·49	50	0·51	0·51
76	0·81	0·81	55	0·57	0·57
92	0·95	0·74	57	0·59	0·48
116	1·19	0·68	76	0·78	0·43
			90	0·92	0·40
	Run off at 78 exposures.		100	1·03	0·41
				Run off at 56 exposures.	

nitric acid. After organic matter had been destroyed by evaporation with sulphuric acid on a sand bath, the copper was estimated colorimetrically by the potassium ferrocyanide method used by Martin (4).

Typical results, quoted in Table I, show that there is no preferential retention of the solid phase and the initial retention can be calculated from the volume of spray necessary to cause incipient run-off or drain-off.

(4) *The Estimation of Tenacity.*—It appears, on *a priori* grounds, impossible to deduce an absolute figure for tenacity, for the persistence of the solid phase remaining upon the surface after a given leaching treatment will be dependent upon the severity of that treatment, e.g. the amount of wash water applied and the force of application. The procedure adopted was accordingly empirical in character. The surfaces were sprayed with an amount of suspension never exceeding that required for run-off or drain-off. After drying for twenty-four hours at room temperatures the plate was refixed in the same position as when sprayed, the pendulum removed, and the deposit drenched with distilled water from the atomiser for one minute at an air pressure of 1·5 atmospheres. The deposit still remaining upon the surface was washed off with nitric acid and the amount of copper present determined by the method given above. The percentage still remaining of the total amount applied was termed the tenacity.

Because the retention of copper and the amounts of copper remaining after this leaching treatment were small, the area of the cellulose nitrate and glyptal resin surfaces exposed to the original spray was increased. The glass screen used in such cases had an aperture $4{\cdot}5 \times 3{\cdot}0$ cm. (= 2·1 sq. inches) instead of 1 square inch.

Experimental Results.

(1) *Analytical Data.*

Of the wide range of materials of potential value as spray supplements, a selection was made on the basis of the structural classification outlined by Evans and Martin (1). Of the long chain aliphatic derivatives, three were chosen in which the long chain is in the anion of the active constituent, viz. Sulphonated Lorol and C32, being of the fatty alcohol sulphate group, and Igepon T, being a fatty acid derivative. Of the derivatives of which the long chain is contained in the kation, Sapamine MS and C30 were selected, whilst, of the un-ionized surface active long chain derivatives, a water-soluble polyglycerol ester (U75) was employed.

Agral 2 and sulphite lye were selected of the spray supplements containing active constituents of cyclic structure, and of the miscellaneous unclassified "spreaders", gelatine, casein, saponin, a water-soluble methyl cellulose and a water-soluble synthetic resin were used.

The transition from simple solutions to suspensions enabled a number of supplements incompletely soluble in water to be used, e.g. lime casein, which has for long been employed in practice in combination with protectant spray materials. Trials were also made of various clays such as Fuller's Earth and bentonite, which have been suggested as spray supplements.

The value of glyceride oils as stickers for Bordeaux mixture was established by Martin (4) and, in America, petroleum oil emulsions are frequently employed as supplements for arsenical sprays applied against codling moth. Although the factors influencing spray retention in spray systems of two non-miscible liquids and a solid have not yet been examined in the laboratory, it was considered advisable to include oil emulsions in the present tests. Two such emulsions were used, and preliminary estimates of the effect of hydrocarbon oil emulsions upon the initial retention and tenacity of cuprous iodide and oxide were made in the laboratory using the method employed for simple suspensions.

Since many of the products mentioned above were from the same bulk sample as that used by Evans and Martin, it is unnecessary to give, in full, the analytical details on which the estimates of content of active constituents were based. Brief particulars of the character and amount of the active ingredients of each sample are as follows:—

Sulphonated Lorol.—This contains approximately 45-7% sodium "lorol" sulphate, "lorol" being a mixture of fatty alcohols in which dodecyl (lauryl) alcohol, $CH_3(CH_2)_{10}CH_2OH$, predominates; the chief concomitant material is the sodium salt of a sulphonated hydrogenated cyclic hydrocarbon.

C32.—This product, stated by the manufacturers to be a powder preparation of the product C72 (1), yielded, on acid hydrolysis, 27·5% ether-soluble fatty alcohol and 4·09% sulphur in the form of sulphate. The content of cetyl sodium sulphate may therefore be assessed at 39-40%. No indications were obtained of the presence of true sulphonates, as in C72. As the ash amounted only to 11·7% the concomitant material appears to be a water-soluble organic derivative.

Igepon T.—The figures quoted by Evans and Martin indicate that the sample contains about 40% taurine-oleic acid condensation product, the concomitant materials being mainly sodium chloride and sodium phosphate.

Sapamine MS.—The solution contains 0·59 gm. nitrogen per 100 ml. and gave, on prolonged acid hydrolysis, 4·8 gm. fatty acid (neutralization value 198) per 100 ml. These figures suggest that the preparation contains from 7·5-9% active constituent which is probably a quaternary ammonium sulphate of Sapamine Base, a condensation product of oleic acid (neutralization value 199) and a substituted ethylene diamine (Hartmann and Kagi, 2), the diluent being water.

C30.—This powder, described by the makers as a long chain alkyl quaternary ammonium bromide, gave 23·6% bromine and only 2·8% ash. It is therefore probable that the content of active constituent approaches 100%.

U75.—This product, of the consistency and appearance of soft soap, was described by the manufacturers as a polyglycerol ester containing about 66% active constituent, the remainder being water.

Agral 2.—For reasons given by Evans and Martin, this product was considered to contain approximately 75% alkylated naphthalene sulphonates as the active constituents.

Sulphite Lye.—Olive and Partington 60° Tw. syrup.

Casein.—A commercial Kahlbaum sample was mixed, before use, with certain proportions of hydrated lime. In addition, a soluble casein (Hopkin and Williams), probably containing the sodium salts of casein degradation products, was used.

Gelatine.—Coignet Extra.

Methyl Cellulose.—Purchased, as a water-soluble product, through a wholesale chemist.

C33.—A white powder, described by the makers as a water-soluble synthetic resin. It proved incompletely soluble in water and was therefore used in but few tests.

Fuller's Earth.—From laboratory stock, purchased through wholesale chemists.

Bentonite.—Both the ordinary commercial bentonite and the grade known as Wil-kinite, selected for detergent and emulsification purposes, were employed.

Cottonseed Oil Emulsion.—Two volumes of a cheap grade of edible cottonseed oil were emulsified in a laboratory mill with one volume of 20% sulphite lye (60° Tw.).

Petroleum Oil Emulsion.—The emulsion was prepared in the same manner as the cottonseed oil emulsion but, instead of the glyceride oil, two volumes of a highly refined (Grade G, 5) petroleum oil were used.

(2) *Initial Retention and Tenacity.*

The results of the systematic survey of spray supplements are assembled in Table II, the solid phase of the suspension being cuprous iodide and the surface paraffin wax. The tenacity figures are expressed as the mean percentage of six or seven replications and the standard error of the mean. Although theoretically a tenacity greater than 100% is impossible, experimental error is, in one case, responsible for a figure exceeding this limit. With gelatine and lime casein, the spray deposit was left as a film which was visibly unaffected by the leaching treatment.

TABLE II.

The Retention and Tenacity of Cuprous Iodide on a Paraffin Wax Surface.

Spray Supplement.	Conc. %	Initial Retention mg. Cu_2I_2/sq. inch.	Tenacity = % initial retention surviving leaching.
Sulphonated Lorol	0·5	1·39	25 ± 4·6
	0·1	1·61	42 ± 3·1
	0·05	1·75	59 ± 2·1
	0·025	1·84	65 ± 5·9
C32	0·5	0·67	45 ± 6·5
	0·05	1·39	88 ± 6·5
Igepon T	0·5	(0·63)	40 ± 7·3
	0·1	(0·85)	64 ± 1·9
	0·05	0·97	90 ± 1·9
	0·025	1·06	100 ± 4·1
Sapamine MS	0·5	0·89	71 ± 2·9
	0·1	0·97	87 ± 2·4
	0·05	1·12	95 ± 5·1
C30	0·5	0·87	95 ± 3·5
	0·1	0·71	100 ± 6·3
U75	0·5	0·50	49 ± 3·9
	0·1	0·69	75 ± 5·4
	0·05	0·75	81 ± 9·4
	0·025	0·94	85 ± 8·6
Agral 2	0·5	1·41	35 ± 2·7
	0·1	1·90	52 ± 2·2
	0·05	2·02	77 ± 3·3
	0·025	2·01	95 ± 5·0
Sulphite Lye	1·0	3·12	10 ± 0·5
	0·5	3·25	63 ± 3·3
	0·1	1·97	65 ± 5·2
	0·05	(1·99)	82 ± 8·2
Gelatine	0·5	1·59	100 —
	0·1	1·34	100 —
	0·05	1·65	100 —
Lime Casein	0·5	0·91	100 —
	0·1	1·45	100 —
	0·05	1·72	100 —
	0·025	2·34	100 —
Saponin	0·5	0·48	68 ± 4·5
	0·1	(0·54)	95 ± 9·2
	0·05	0·68	104 ± 5·5
	0·025	(3·10)	100 ± 10·0
Methyl Cellulose	0·5	0·86	27 ± 5·7
	0·1	0·76	77 ± 2·5
	0·05	0·80	78 ± 2·5
	0·025	0·80	95 ± 3·5
Nil	—	2·10	100 ± 3·0

TABLE III.

The Retention and Tenacity of Cuprous Iodide on a Cellulose Nitrate Surface.

Spray Supplement.	Conc. %	Initial Retention mg. Cu_2I_2/sq. inch.	Tenacity = % initial retention surviving leaching.
Sulphonated Lorol	0·2	0·30	5 ± 0·5
	0·05	0·45	14 ± 2·3
	0·025	0·73	53 ± 2·0
C32	0·2	0·29	39 ± 1·7
	0·05	0·32	70 ± 5·0
	0·025	0·55	84 ± 2·9
Igepon T	0·2	0·22	52 ± 4·0
	0·05	0·31	57 ± 0·9
	0·025	0·42	68 ± 4·6
Agral 2	0·2	0·35	11 ± 2·2
	0·05	0·95	61 ± 6·7
	0·025	1·42	73 ± 5·4
Sulphite Lye	0·5	0·46	2 ± 0·8
	0·1	1·11	3 ± 0·9
	0·05	(1·44)	7 ± 2·9
	0·025	(1·63)	9 ± 2·8
Gelatine	0·05	0·39	88 ± 2·8
	0·025	0·47	76 ± 4·7
	0·01	(1·2)	79 ± 5·9
Casein (soluble)	0·1	—	90 ± 0·9
	0·05	—	91 ± 6·7
	0·025	—	90 ± 4·2
	0·01	—	76 ± 4·3
Methyl cellulose	0·05	0·51	23 ± 3·8
	0·025	0·47	9 ± 2·5
	0·01	(0·50)	29 ± 1·2
	0·005	0·63	50 ± 1·4
C33	0·2	—	90 ± 3·1
	0·025	—	97 ± 6·1
Fuller's earth	1·0	1·91	7 ± 2·4
Bentonite	1·0	1·91	45 ± 6·3
Wil-kinite	1·0	1·91	17 ± 4·2
Nil	—	1·91	28 ± 3·0
Lime (hydrated)	1·0	1·91	23 ± 1·9
Petroleum oil emulsion	1·0	1·97	78 ± 2·7

Table III contains the results obtained on the cellulose nitrate surface whilst Table IV shows the results with the glyptal resin surface. In the last-named series the solid phase of the suspension was red cuprous oxide.

Table IV.

The Retention and Tenacity of Cuprous Oxide on the Glyptal Resin Surface.

Spray Supplement.	Conc. %	Initial Retention mg. Cu_2O/sq. inch.	Tenacity %
Sulphite Lye	0·75	0·73	65 ± 3·0
Methyl cellulose	0·005	0·38	86 ± 2·9
Sulphonated Lorol	0·05	0·27	87 ± 3·3
Lime	1·0	0·69	87 ± 2·9
Agral 2	0·05	0·33	91 ± 3·0
Nil	—	0·92	92 ± 3·8
Gelatine	0·1	0·29	100 ± 1·9
Petroleum oil emulsion	1·0	0·68	101 ± 2·1
Lime Casein (Lime : Cas. 7 : 1)	0·25	0·26	101 ± 3·0

Table V.

Number of Exposures necessary to give Excess of Spray.

Surface.	Spray.		Number of exposures.	Mean.
Paraffin	Water	Alone	80; 72; 77; 94; 87; 78	81·0
		+Cu_2I_2	90; 72; 85; 80; 77	80·8
,,	0·5% Sulph. Lorol	Alone	44; 46; 45	45·0
		+Cu_2I_2	45; 48; 48; 46	46·8
,,	0·5% Sapamine	Alone	29; 30; 29	29·3
		+Cu_2I_2	28; 30; 30	29·3
,,	1·0% Sulphite Lye	Alone	85; 88; 84; 84	85·2
		+Cu_2I_2	84; 86; 83; 87	85·0
,,	Lime Casein (0·05% casein)	Alone	18; 20; 19	19·0
		+Cu_2O	19; 19; 20	19·3
Cellulose Nitrate	0·5% Methyl cellulose	Alone	13; 13; 13	13·0
		+Cu_2I_2	13; 13	13·0
Glyptal resin	0·005% Methyl cellulose	Alone	16; 16; 16	16·0
		+Cu_2O	16; 15; 16	15·7
,,	Lime Casein (0·05% casein)	Alone	12; 11; 12; 11	11·5
		+Cu_2O	12; 12; 11; 11	11·5

Discussion.

Initial Retention.—In Part I of this series, Evans and Martin showed that the maximum amount of spray initially retained upon a plane surface is determined by wetting and spreading properties, markedly decreasing with increase of these properties. It was anticipated that the addition of the suspended particles might modify the wetting and spreading properties of the aqueous phase and thereby affect spray retention. This anticipation was not always realized and, in the majority of cases, the presence of the solid did not alter the volume of spray required to achieve run-off or drain-off. In Table V, for example, the number of exposures required to attain excess of spray with and without cuprous iodide and red cuprous oxide, respectively, are recorded.

In only a few of the systems examined did the presence of the solid affect initial retention. The results obtained with these systems are given in Table VI, in which the number of exposures required to give run-off are recorded and the weight of spray retained is calculated from the average retention figure for water on paraffin wax (=210 mg. per sq. inch).

Table VI.

Abnormal Retention at Run-off.

System.	Conc. of supplement %	No. of exposures to give run-off.		Retention mg./sq. inch.	
		Aq. sol.	Cu_2I_2 suspension.	Aq. sol.	Cu_2I_2 suspension.
Gelatine/ paraffin surface	0·5	80	50	255	159
	0·1	64	42	204	134
	0·05	116	52	369	165
	0·025	146	74	465	235
	0·000	86	66	(210)	(210)
Methyl cellulose/ paraffin surface	0·5	65	43	130	86
	0·1	53	38	106	76
	0·05	52	40	104	80
	0·025	53	40	106	80
	0·01	60	49	120	98
	0·005	69	59	138	118
	0·000	105	—	(210)	—
			Cu_2O suspension.		Cu_2O suspension.
Sulphite Lye/ Glyptal surface	0·75	24	20	118	98
Petroleum oil emulsion/ Glyptal surface	1·0	37	31	181	152

With the glyptal surface it was found that the addition of hydrated lime modified the degree of retention of water. Thus, the retention of water alone was 204 mg. per sq. inch, whereas that of the lime suspension was 152 mg. per sq. inch. No detailed examination of this phenomenon was made but in this case and in those in which the addition of cuprous iodide or cuprous oxide affected retention, it was observed that the addition of the solid particles visibly affected the wetting properties of the spray, for the droplets exhibited a lower contact angle than the simple aqueous solution. It may be noted that the effect was not observed upon the cellulose nitrate surface. Here the effect may have been masked by experimental error but the observation suggests that the nature of the solid surface should not be neglected in the subsequent investigation of the phenomenon.

The observation that, in the majority of systems, the addition of the solid phase was without influence upon initial retention enabled a simplification of the laboratory work, for it became necessary only to establish, at the highest concentration of supplement used, that the addition of the solid particles did not change the number of exposures required for run-off. If this proved to be the case, it could be assumed that the retentions of suspensions containing lower concentrations of the spray supplement were identical with those of the aqueous solution of the supplement. As this figure was known from previous work, the amount of copper retained upon the surface could be calculated. This amount is recorded in Tables II and III as mg. cuprous iodide per sq. inch.

In view of the close similarity between the retention of suspensions and the retention of the aqueous phase alone, further comment on the figures may be postponed until a more detailed examination of the correlations between retention and wetting and spreading properties has been made. It may, however, be noted that for those systems in which the presence of the solid particles has no influence upon retention, it follows that particle size, up to the maximum used in the tests, is not a factor influencing initial retention. This corollary is naturally not applicable to those surfaces encountered in practice on which the presence of hairs or fungal threads leads to an accumulation of solid through filtration. It does, however, indicate that particle size exerts its influence upon the efficiency of a protectant spray more through its effect upon coverage than upon initial retention.

Tenacity.—The systems under examination are of three components, the liquid, the solid particle and the surface. The interdependence of these three variables in affecting the tenacity of the spray deposit is well shown by cuprous iodide in water suspension. On the paraffin wax surface the spray deposit was unaffected by the leaching treatment, whereas, on the cellulose nitrate, only 28% survived. This difference is probably explained by the observation that,

on the cellulose nitrate surface, the cuprous iodide deposit shrank during drying, a process which no doubt renders the deposit less tenacious. Shrinkage did not occur either on the paraffin surface or with the more crystalline cuprous oxide particles. Moreover it was prevented by the addition of small amounts of supplements and, in such cases, even highly surface-active wetting-out agents in small amount improved tenacity, thereby functioning as true "stickers". The only exceptions observed were Fuller's Earth and Wil-kinite, the deleterious effect of which on tenacity seems to be due to an enhancement of this colloidal shrinkage.

Where shrinkage was not a disturbing factor it was generally found that the addition of supplements of high surface activity decreased tenacity; with these supplements (e.g. Sulphonated Lorol, C32, Igepon T, Sapamine MS, C30, U75, Agral 2 and Saponin) the reduction of tenacity became greater as the concentration of supplement was increased. As the results supply no indication that the molecular structure of the supplement influences the degree of reduction of tenacity, the probable explanation of this action is that the presence of a wetting agent in the spray residue renders it more easily wetted by the leaching water, resulting in a greater loss of the deposit. The relatively greater decrease in tenacity of cuprous iodide upon cellulose nitrate as compared with that on paraffin wax is in accordance with this hypothesis. Sulphite lye is outstanding in its deleterious effect upon tenacity, a property which may be ascribed to its exceptional powers as a dispersing and emulsifying agent. It is feasible to suppose that those properties which promote dispersion will function in the spray residue dispersing it in the leaching water and thereby reducing tenacity.

Of the supplements which were added primarily as "stickers", e.g. gelatine, lime casein and methyl cellulose, the first two show a favourable effect on tenacity. All three supplements yield suspensions which, on paraffin wax, show the type of drain-off designated (a) by Evans and Martin, an indication of high wetting properties. The apparent improvement in wetting properties induced by the presence of solid particles in solutions of gelatine and methyl cellulose has already been illustrated by Table VI. On drying, however, the films are no longer readily wetted by the leaching water, and the removal of spray residue is therefore low. With methyl cellulose, which, even at the lowest concentrations used imparted good wetting properties to the suspensions, the low tenacity may be associated with its relatively greater solubility in the leaching water. On this hypothesis it may be concluded that for a spray supplement to exhibit good wetting and spreading properties and yet have no unfavourable influence upon tenacity it should, after application, dry to a deposit not easily wetted by water.

FIELD TRIALS.

In order to check the conclusions of the laboratory tests by field trials under practical conditions, it was decided to select a crop plant on which the efficiencies of the protectant sprays could simultaneously be determined by biological assay. Of the possible crop plants, potato was chosen as that most suitable, for the following reasons: (1), the efficiencies of the various sprays as protectants against Blight (*Phytophthora infestans*) could be compared, provided the disease appeared in the plots or in the unsprayed control plants; (2), attack by this fungus usually occurs after the rapid initial development of leaf surface has ceased and, accordingly, errors due to the presence of unsprayed new growth are reduced; (3), the potato is, by virtue of its low habit of growth, easy to spray, the foliage is easily sampled and the degree of Blight infestation relatively easy to record; (4), the acreage required for the trials is small; (5), the provision of a suitable supply of test material offers no difficulty.

Experimental Methods.

(1) *Lay-out of Trial.*—The area available for the test was a plantation of pears interplanted within the rows with red currants and laid out in 1935. A double row of King Edward potatoes, a variety chosen because of its susceptibility to Blight, was planted between each pear row. Scotch seed was used to reduce the complications of disease recording and foliage sampling caused by virus diseases.

Each of the thirteen double rows was subdivided into lengths of ten paces, making 130 individual plots. The spray treatments were distributed so that each of the ten blocks, one block running at right angles to the direction of the rows, constituted a randomized block.

(2) *Sprays used.*—Of the thirteen spray treatments, only ten were concerned with the influence of spray supplements upon protective fungicidal properties. The protective fungicide used was a paste preparation of yellow cuprous oxide (35% Cu). To prevent sedimentation to an unworkable deposit during storage a minimum of protective colloid, 1·9% powdered sulphite lye, was incorporated in the preparation whilst, to overcome frothing during manufacture, a small amount (0·2%) of a proprietary anti-foaming agent was added.

The spray supplements tested were added to the cuprous oxide spray (containing 0·2% metallic copper) at the following rates:—

Treatment 6: Sulphonated Lorol, 8 oz. per 100 gal.
,, 5: Agral 2, 8 oz. per 100 gal.
,, 3: Sulphite lye, 6 pints syrup (60° Tw) per 100 gal. (=approx. 1%).

Treatment 10: Gelatine, 1 lb. per 100 gal.
,, 2: Lime casein, 10 lb. hydrated lime and 8 oz. casein per 100 gal.
,, 1: Lime, 10 lb. hydrated lime per 100 gal.
,, 4: Methyl cellulose, 0·8 oz. per 100 gal. (0·005%).
,, 8: Cotton seed oil emulsion, 1 gal. (=0·67 gal. cotton seed oil and 0·33 gal. 20% sulphite lye) per 100 gal.
,, 9: Petroleum oil emulsion, 1 gal. (=0·67 gal. oil and 0·33 gal. 20% sulphite lye) per 100 gal.
,, 7: No added supplement.

Particulars of the various products used are given on pp. 5 and 6.

(3) *Spray application.*—To enable comparisons to be made between all treatments it was necessary to complete each application in one day. As one hundred widely-distributed plots had to be sprayed the most rapid method possible was required. Further, to eliminate variation in spray retention due to uneven application, each plot had to receive equal amounts of spray. Despite the apparent departure from practical spraying methods, the one selected was by means of watering cans, each plot receiving the contents of a 1 gal. can. In view of the relative ease with which potato foliage is wetted, it was considered improbable that the force of application, which might be of importance with a solid/liquid system exhibiting a high receding contact angle, would affect spray retention. Further, the emulsions, the stability of which might be enhanced by passage through spray pumps and nozzles, were initially of a high degree of dispersion and stability.

The first application was begun on July 22nd and treatments 3, 4, 5 and 8 were applied under perfect conditions. Early rain on July 23rd prevented prompt completion of the application, and soon after treatment 10 had been applied a slight shower fell before the spray had dried on the foliage. It was considered possible, but improbable, that the shower had affected spray residue. The second application was made under good conditions on August 7th.

(4) *Estimates of Spray Residue.*—For the estimation of the amount of copper protectant remaining on the foliage throughout the trial, periodic samplings of the foliage were made. Twenty leaflets were collected at random from each plot and the ten samples per treatment were mixed in the laboratory. From each leaflet, a disc of 1·325 cm. diameter was cut by means of a cork borer, care being taken to avoid the edges and tips of the leaflets. The four samples of fifty discs per treatment were ashed separately and the copper present was determined by the colorimetric ferrocyanide method.

(5) *Estimation of Blight Infestation.*—Previous but, as yet, unrecorded experience of the estimation of Blight infestation has indicated that a suitable

method is to assess the degree of prevalence of the disease on the foliage of each plot which is given an estimate based upon the following scale:—

0 = No Blight apparent.
2 = Blight lesions scarce.
4 = ,, ,, occasional.
6 = ,, ,, frequent.
8 = ,, ,, ,, with defoliation.
10 = Haulm completely defoliated.

The odd numbers were used for intermediate degrees of Blight infestation.

Experimental Results.

(1) *Estimates of Spray Residue.*—Foliage sampling was carried out as soon as possible after each application in order that the figure might give a measure of initial retention. Unfortunately, frequent showers during and after the first application caused delay and sampling was not completed until July 25th. The second sampling was made on August 5th, just prior to the second application. Following that application no rain fell until after the third sampling was completed on August 11th. The fourth and final sampling was made on August 27th, by which time the disease had caused such serious defoliation that no further samples were taken, for it was feared that Blight might have preferentially attacked those leaflets carrying a small proportion of spray residue and thereby prevented true sampling.

Table VII.

Estimates of Copper Retention.

Treatment	1	2	3	4	5	6	7	8	9	10
Date of Sampling										
24 & 25.7.36	4·8	3·3	4·2	5·0	4·5	5·3	8·2	4·2	6·8	4·3
	7·8	3·0	4·5	5·5	4·0	4·8	5·2	6·4	6·1	2·7
	8·4	3·5	5·2	5·6	4·2	4·6	5·5	7·6	7·2	3·8
	7·4	3·7	5·6	4·2	—	4·2	6·2	6·6	6·7	3·8
5.8.36	4·5	2·4	2·3	2·8	3·6	3·5	3·2	3·6	4·6	2·4
	3·5	3·1	2·2	4·5	3·5	4·3	3·2	3·2	4·2	2·7
	4·7	2·5	2·0	2·1	3·0	2·3	3·2	4·8	5·4	2·3
	—	2·6	2·2	3·2	3·1	2·5	3·4	4·2	4·3	3·3
11.8.36	14·4	12·4	13·8	13·4	6·1	14·0	14·8	18·2	24·4	11·0
	20·0	10·4	15·2	16·4	8·5	12·0	14·0	21·2	27·8	13·8
	12·4	8·2	20·4	16·0	6·0	14·0	12·0	18·2	26·0	16·8
	16·8	14·0	18·0	12·0	8·0	10·0	15·6	20·2	27·6	12·8
27.8.36	12·8	6·6	8·0	9·6	6·1	7·0	10·3	16·4	24·2	8·8
	9·4	9·4	9·0	10·0	5·8	7·4	8·2	15·6	20·0	8·4
	9·4	8·4	9·4	9·8	7·6	7·8	9·8	20·6	27·4	10·0
	8·6	10·2	10·4	9·6	5·0	9·2	12·2	18·8	24·0	8·0

The analytical results are recorded in Table VII which gives the amount of copper expressed as mg. $Cu \times 10^{-1}$ per 50 leaf discs of diameter 1·325 cm., found in each sample. The analysis of variance of these results is summarized in Table IX.

TABLE VIII.

Estimates of Blight Infestation.

Treatment	1	2	3	4	5	6	7	8	9	10
Block I	2	2	2	1	2	3	2	3	2	2
	3	3	3	3	3	4	3	3	3	4
	4	4	5	4	5	5	4	4	5	5
	6	6	8	7	7	8	7	7	6	7
Block II	2	3	3	2	3	2	2	2	3	1
	3	4	4	4	4	3	4	3	4	4
	5	6	5	5	6	4	6	4	5	4
	8	8	8	7	8	8	8	7	6	7
Block III	1	3	2	2	2	3	3	1	2	2
	3	5	4	3	4	5	4	3	3	3
	4	6	5	5	5	6	6	3	4	5
	7	9	8	8	7	8	8	7	6	8
Block IV	2	1	2	2	2	3	3	3	1	3
	4	3	3	5	3	5	4	6	3	4
	6	4	4	3	6	6	6	7	4	6
	9	7	7	7	8	9	8	9	6	9
Block V	2	2	2	3	3	2	3	3	2	3
	3	3	3	4	4	4	5	5	4	5
	4	5	5	5	6	5	7	7	6	6
	7	7	8	8	8	8	9	8	8	9
Block VI	3	1	2	2	3	2	3	3	3	2
	4	4	4	4	5	4	5	4	4	4
	5	5	5	6	7	5	6	5	5	5
	7	9	8	8	9	8	8	8	7	8
Block VII	2	1	3	3	3	2	3	3	2	3
	4	4	4	5	5	4	5	4	4	5
	5	6	6	6	6	5	6	5	6	6
	7	9	8	9	9	8	9	8	8	9
Block VIII	2	3	2	2	3	3	2	2	3	4
	5	5	4	4	5	4	3	4	5	6
	6	6	5	6	7	5	5	6	7	7
	9	9	8	8	9	7	7	8	9	9
Block IX	2	2	2	3	4	2	3	3	3	3
	5	3	4	5	6	4	5	5	5	5
	6	4	4	7	7	6	7	7	6	6
	8	6	7	9	9	8	9	9	8	9
Block X	4	3	3	3	3	3	3	2	2	3
	6	5	4	5	4	4	5	4	3	5
	7	7	6	7	5	5	7	5	5	6
	9	9	9	10	7	7	10	7	7	9

(2) *Estimates of Blight Infestation.*—On July 22nd, prior to the first application, the degree of Blight infestation was uniform throughout the plots and was estimated as 1. The remaining estimates are given in Table VIII, the estimates for each plot made on July 28th, August 6th, 17th and 29th being recorded in this order vertically for each plot. On the final date, it was decided that, as the estimates on many plots approached the limit of 10, further estimates would not supply useful data. The estimates were submitted to statistical analysis with the results given in Table X. In both Tables IX and X the results received for the remaining three spray treatments are included.

TABLE IX.

Statistical Analysis of Estimates of Spray Residue.

	Degrees of Freedom.	Sum of Squares of Deviations.	Variance.
Treatments ..	12	1,914·85	159·57
Dates	3	5,054·88	—
Remainder	188	1,414·43	7·52

TABLE X.

Statistical Analysis of Estimates of Blight Infestation.

	Degrees of Freedom.	Sum of Squares of Deviations.	Variance.
Treatments (T) ..	12	38·0	3·167
Blocks (B)	9	87·0	—
Dates (D)	3	2,138·8	—
Interactions :			
T×B	108	188·0	—
B×D	27	8·7	—
T×D	36	10·4	—
Remainder	324	77·1	0·238

DISCUSSION.

For the discussion of the field results and their concordance with laboratory results, it is convenient to analyse the data on the basis proposed in the introductory section of the present paper. It is there suggested that protective efficiency is determined by two main sets of factors, the availability and the spray residue factors, the latter being the resultant of the factors influencing initial retention and those determining tenacity.

THE INFLUENCE OF AVAILABILITY FACTORS.

To separate the influence of these factors from the effects of spray residue upon the efficiency of the protectant, the degree of control as measured by the

average Blight estimate for each treatment may be compared with the spray residue on the assumption that the mean figure for copper deposit represents a summation of the amounts of copper present throughout the period of the test. This assumption implies that the mean copper retention figure represents the average amount of material capable of being rendered fungicidal and present on the foliage at each time when environmental conditions favour infection.

TABLE XI.

Comparison of Blight Infestation and Copper Residue.

Spray supplement.	Mean of 40 Blight estimates.	Spray residue.	Mean of copper estimates.	No. of estimates.
Petroleum oil	4·625	Petroleum oil	15·42	16
Sulphite lye	4·725	Cottonseed oil	11·86	16
Lime	4·775	Lime	9·32	15
Lime casein	4·800	Nil	8·44	16
Cottonseed oil, Sulphonated Lorol	4·925	Sulphite lye	8·31	16
		Methyl cellulose	8·11	16
Methyl cellulose	5·000	Gelatine	7·18	16
Gelatine	5·275	Sulphonated Lorol	7·06	16
Agral 2	5·200	Lime casein	6·48	16
Nil	5·325	Agral 2	5·25	15
Significant Difference (P=0·05)	0·222	between means of 16 estimates		1·98
		between means of 15 and 16 estimates		2·02
		between means of 15 estimates		2·08

In Table XI, the various treatments are arranged in the first column in order of protective efficiency as judged by the average Blight estimate per treatment and, in the third column, in order of the average copper residue. A comparison of the two orders of merit reveals a high degree of correlation and, in the cases of non-agreement, it is possible to advance tentative explanations. The fungicidal efficiency of cuprous oxide in the absence of a spray supplement (the 0·01% sulphite lye derived from the preparation may be considered negligible) is lower than would be expected from the mean copper residue, an indication that the addition of the supplement has induced a more uniform distribution of the copper compound and, thereby, enhanced in a sense its availability. This effect might account for the improvement of protectant qualities conferred by sulphite lye, lime casein, Sulphonated Lorol and Agral 2. The relatively low position of the cotton seed oil spray in order of fungicidal efficiency as compared with its position in the third column suggests that cotton seed oil has in some way, perhaps by virtue of its small content of free fatty acid, affected availability in a manner not definitely shown by the petroleum oil. Further, as no reduction of fungicidal activity is observable when casein (as lime casein) or gelatine is added, it may be concluded that these proteins do not reduce the

availability of the copper fungicide, an observation of interest in connection with the hypothesis that the toxic action of copper compounds is associated with a combination with, or an adsorption by, the protein of the organism. If this were the mode of action of the copper fungicides, it would seem that the presence of extraneous protein would reduce activity, an effect not observed in the above trials.

The Influence of the Supplement on Initial Retention.

Proceeding with the analysis of the copper residue estimates, the initial retention may be considered to be represented approximately by the copper estimates of the first foliage samplings after each spray application. Neither in the sample taken on July 24th/25th nor in that of August 11th does the figure supply a true measure of spray retention for, on the first occasion, rain intervened between spraying and sampling, whilst the figure obtained on the second date included spray residue from the first application. In Table XII, the various treatments are arranged in each column in the order of spray residue.

Table XII.

Initial Retention of Copper on Foliage.

First Application.			Second Application.	
Spray Supplement.	Mean copper estimate.	No. of estimates.	Spray Supplement.	Mean of four copper estimates.
Lime	7·10	4	Petroleum oil	26·25
Petroleum oil	6·70	4	Cottonseed oil	19·35
Nil	6·27	4	Sulphite lye	16·80
Cottonseed oil	6·20	4	Lime	15·90
Methyl cellulose	5·07	4	Methyl cellulose	14·45
Sulphite lye	4·87	4	Nil	14·10
Sulphonated Lorol	4·72	4	Gelatine	13·60
Agral 2	4·23	3	Sulphonated Lorol	12·50
Gelatine	3·65	4	Lime casein	11·25
Lime casein	3·37	4	Agral 2	7·15
Significant difference	1·50	4		4·27
(P=0·05)	1·70	3 and 4		

The large difference required for significance is doubtless responsible for many of the apparent inconsistencies in the two relative orders, but interesting points of agreement are shown. Noteworthy are the high positions of the oils and the low positions of Sulphonated Lorol and Agral 2, supplements of high surface activity. Lime casein is, in both columns, low as compared with lime, whereas gelatine is lower than might be expected by reason of its high viscosity

which, as Woodman (6) has stated, would cause an increase in the volume of spray retained by leaves in spraying.

No strict comparison of the field results and the laboratory results on initial retention can be made, for the latter represent the maxima which can be attained. Further, in the laboratory, the surfaces are held at right angles to the direction of the spray, under which conditions the total volume applied is retained until the maximum is reached. In the field the leaf surface is rarely, if ever, perpendicular to the direction of the spray and, under such conditions, only a fraction of the total spray applied is retained, the magnitude of the fraction being related, in a manner as yet uninvestigated, to the wetting and spreading properties of the spray on the foliage concerned. To illustrate this point, certain results obtained by Mr. B. Collie in this laboratory may be quoted. In these experiments the surface was tilted at 45° to the direction of the spray and the volume required to produce run-off was determined.

TABLE XIII.

Fraction of Spray retained on Inclined Surface.

	Paraffin Wax.				Cellulose Acetate.			
Conc. %	0	0·1	0·5	1·0	0	0·1	0·5	1·0
Igepon T	0·63	0·67	0·65	0·74	0·65	0·70	0·72	0·81
Agral 2	0·66	0·68	0·69	0·75	0·63	0·66	0·68	0·81
Sulphonated Lorol	0·60	0·66	0·66	0·72	—	0·62	0·65	0·74
C32	0·60	0·64	0·63	0·71	0·64	0·65	0·66	0·76

In Table XIII, the volume of spray retained per unit area of tilted surface is expressed as a fraction of the actual amount applied per unit area of surface perpendicular to the axis of the spray cone. As the area exposed to the spray is increased by tilting, the theoretical ratio indicating complete retention is sin. 45° = 0·71. That this figure is sometimes exceeded is due, in part, to the nearer approach of part of the tilted surface to the spray nozzle. The figures show that, with water and the lower concentrations of supplements, part of the volume of spray applied is not retained. Consequently it may be assumed that of the first amounts of spray to reach the foliage, the proportion retained increases with the wetting and spreading properties of the spray. The maximum retention figure for each particular spray is therefore more rapidly attained with sprays of good wetting and spreading properties. Further, with surfaces held perpendicular to the spray, the initial retention figures are lower with liquid/solid systems exhibiting low contact angles. For comparison with field results in which no great excess of spray was applied, it is, for these reasons better to select laboratory results obtained on a readily wetted surface.

In Table XIV, the supplements are arranged in order of initial retention on paraffin wax, cellulose and glyptal resin surfaces, the concentration of each supplement being that used in the field trials, with the exception of lime casein (0·25% 1:4 mixture of hydrated lime) on the glyptal resin surface. The results show, as indicated in Table XIV, that the maxima are relatively lower on the more readily wetted cellulose nitrate surface than on the paraffin wax. For comparison with the field results given in Table XII, the cellulose nitrate surface, which incidentally approximates to potato foliage in relative ease of wetting, is selected. On this surface, the oil, no supplement, and lime occupy the high positions, whereas gelatine and lime casein are low, as in the field trials. The most marked exception is Agral 2 which gives a relatively greater initial retention in the laboratory than in the field. Taking into consideration the various factors limiting complete concordance between laboratory and field results, the degree of agreement is encouragingly high.

The Effect of the Supplement on Tenacity.

To represent the field estimates of tenacity, the amounts of residue for the second and fourth samples, expressed as percentages of the residue at the first and third samplings respectively, may be taken. These percentages have no absolute significance and the two sets of figures obtained from the two applications will bear no strict proportionality, for each set represents tenacity under different conditions of time and weathering. The figures can therefore be used only to place the various supplements in order of their favourable or unfavourable effect upon tenacity. These orders are given in Table XV in which are included the laboratory figures obtained with cuprous iodide suspensions on the cellulose nitrate surface and with cuprous oxide suspensions on the glyptal resin surface, the concentration of each supplement being that employed in the field trials. No laboratory figures are available for the cottonseed oil emulsion.

Noteworthy among the results shown in Table XV is the favourable effect of lime casein and gelatine upon tenacity, an observation, in the latter case, in agreement with Woodman's (6) prediction that gelatine, being relatively insoluble in cold water, would render the spray deposit less liable to be washed off by rain. The effect of lime casein is in marked contradistinction to that of lime, with which the results are inconsistent with the hypothesis that lime will function as a sticker by virtue of its *in situ* carbonation. The close correlation between the relative positions of the various supplements in the four columns of Table XV is an indication that the laboratory method provides an assessment of effect upon tenacity applicable to field conditions with a high degree of accuracy though, as was expected, the actual percentage figures have no quantitative significance.

TABLE XIV.

Initial Retention on Laboratory Surfaces.

Paraffin Wax.		Cellulose Nitrate.		Glyptal Resin.	
Supplement.	mg. spray per sq. inch.	Supplement.	mg. spray per sq. inch.	Supplement.	mg. spray per sq. inch.
Sulphite Lye	318	Petroleun Oil	186	Nil	204
Gelatine	246	Nil / Lime	180	Lime	152
Petroleum Oil	218			Petroleum Oil	151
Nil / Lime	200	Agral 2	87	Sulphite Lye (0·75%)	98
		Methyl cellulose	62	Methyl cellulose	84
Agral 2	185	Sulphonated Lorol	45	Agral 2	73
Sulphonated Lorol	157	Sulphite Lye	40	Gelatine	64
Methyl cellulose	131	Gelatine / Lime casein	37	Sulphonated Lorol	60
Lime casein	75			Lime casein	58

TABLE XV.

Effect of Supplements upon Tenacity.

Laboratory Figure.				Field Figure.			
Cu_2I_2/cellulose nitrate.		Cu_2O/glyptal resin.		First application.		Second application.	
Lime casein	(93%)	Lime casein / Petroleum oil	(101%)	Lime casein	(77%)	Petroleum oil	(91%)
Gelatine	(88%)			Agral 2	(76%)	Agral 2	(85%)
Petroleum oil	(78%)	Gelatine	(100%)	Gelatine	(73%)	Lime casein	(78%)
Agral 2	(61%)	Nil	(92%)	Petroleum oil	(69%)	Methyl cellulose	(68%)
Methyl cellulose	(50%)	Agral 2	(91%)	Sulphonated Loral	(66%)	Gelatine	(65%)
Nil	(28%)	Lime / Sulphonated Lorol	(87%)	Methyl cellulose	(61%)	Lime	(64%)
Lime	(23%)			Nil	(58%)	Sulphonated Lorol	(63%)
Sulphonated Lorol	(14%)	Methyl cellulose	(86%)	Lime	(54%)	Nil	(62%)
Sulphite Lye	(3%)	Sulphite Lye	(65%)	Sulphite Lye	(45%)	Sulphite Lye	(55%)
				Cottonseed oil	(63%)	Cottonseed oil	(92%)

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SUMMARY.

1. The physical properties of spray deposits affecting the efficiency of protective insecticides and fungicides are discussed. The quantity of spray residue is determined by the initial amount deposited upon the sprayed surface (initial retention) and the ability of the deposit to withstand the action of the various agencies tending to its removal (tenacity). In addition, qualities of the spray deposit such as the uniformity of deposition (coverage) are concerned in relation to their effect upon the action of solubilizing agencies (availability) and upon tenacity.

2. The initial retention of the solid particles of the suspensions examined is shown to be that of the aqueous phase, an indication that no preferential retention of the solid occurs.

3. In the majority of the systems examined, the initial retention of the suspension is the same as that of the aqueous phase but, in a number of cases, the presence of solid particles reduces initial retention. This phenomenon appears to be related to the effect of the solid particles on the wetting and spreading properties of the spray, but it is shown not to be independent of the nature of the surface sprayed.

4. The tenacity of the spray deposit is dependent upon the characters of the spray supplement, of the solid particle and of the surface sprayed. As a general rule, the degree of tenacity is determined by the relative ease of wetting of the spray deposit and, in accordance with this hypothesis, (i) tenacity is comparatively higher upon surfaces wetted with difficulty, (ii) supplements yielding residues insoluble in cold water, e.g. gelatine and lime casein, exert a favourable effect upon tenacity, whereas highly surface-active supplements, especially at high concentrations, exert a deleterious effect upon tenacity, (iii) oils, both hydrocarbon and glyceride, enhance tenacity.

5. The laboratory method for the estimation of initial retention furnishes results in satisfactory agreement with field estimates based upon spray residue determinations.

6. The laboratory estimates permit the arrangement of the spray supplements examined in order of favourable and unfavourable effect upon tenacity. This order is almost identical with that deduced from field trials.

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BACTERIOSIS OF CHERRY TREES:

RELATIVE SUSCEPTIBILITY OF VARIETIES AT EAST MALLING

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THE East Malling collection of cherry varieties was begun early in 1922 with trees of twenty-seven varieties kindly presented by Mr. E. A. Bunyard. Its primary object was the identification and classification of varieties, and particularly the study of varietal habit and behaviour.

The collection has grown rapidly ; at the end of 1936, though a few native and a number of overseas varieties had not yet been obtained, the names represented reached a total of 179 (155 sweet cherry, 12 " Dukes ", and 12 acid cherry). Of these a considerable number have not yet fruited ; it is already known, however, that at least twelve pairs of names are synonymous. On the other hand, at least eight names have to serve for two or more varieties each, no alternative names having yet been found. It is probable that the actual number of varieties in the collection is between 160 and 170.

The disease now known as Bacterial Canker early made its appearance. Within a few years it became evident that varieties differ widely in their susceptibility to the disease ; whilst a large number were moderately affected, some appeared to be almost immune, and others were so highly susceptible that it has hardly yet been possible to carry on a tree to the fruiting stage. A brief summary of the data so far collected, and a comparison with notes made elsewhere, may be of interest.

RELIABILITY OF OBSERVATIONS.

Whilst there can no longer be any shadow of doubt as to the existence of extreme differences in susceptibility between varieties, it is recognized that various factors may make the minor differences observed at East Malling less reliable as a guide to cherry planters. Even though, in the table of susceptibility given below, varieties planted since 1932 are omitted, it is obvious that, of the remainder, the older trees have run much more risk of infection than younger trees. Some trees or scions, also, may already have been infected when received, whilst others were healthy ; and it is plain that minor differences based on observation of not more than two or three trees of a variety cannot be completely trustworthy. When allowance is also made for the possibility, discussed below, that local conditions may influence the relative susceptibility of varieties, it will

not be surprising if the experience of others is in some cases widely different from that at East Malling.

Influence of Local Conditions.

The soil of the four acre plot occupied by the older part of the East Malling variety collection varies considerably, particularly in its depth. This may in part account for the fact that the trees on a strip in the middle have been more severely affected by Bacterial Canker than have those elsewhere. But that this has not greatly affected relative susceptibility is shown in two ways. In the first place, the two trees of White Heart, which have so far proved highly resistant, are in this strip, and have long been surrounded by badly diseased trees. Secondly, in a number of cases, the original tree of a variety was planted on a headland, far from this strip, and the two trees " worked " from it were planted on the strip. In no such case was the apparent susceptibility of the variety much altered.

It is indeed interesting to observe how consistently the two trees of each variety " worked " at East Malling have reacted similarly to Bacterial Canker. As a rule, the two trees have been planted close together ; but where one has died, another has sometimes been planted at a distance. The instances of severe damage to one tree and little to the other are very few, and have nearly all occurred whilst the trees were young ; as time elapses, the reaction of the two trees invariably tends to be more similar. This has occurred even where varieties planted under two names at a considerable distance apart have eventually proved to be identical. In at least one such case, referred to below, a peculiar type of injury from Bacterial Canker led, indeed, to the identification of a variety.

Although the present writer's experience of cherry Bacterial Canker in conditions different from those at East Malling is rather limited, he has observed several instances where groups of varieties have shown a relative susceptibility closely similar to that at East Malling. The exceptions are few, and perhaps merit a short discussion.

The first concerns Géante d'Hedelfingen. This variety, received at East Malling under this name and also as " Bradbourne Black ", has proved extremely susceptible to Bacterial Canker. It has never yet been possible to obtain a head more than three years old from the crotch ; consequently there has been little fruit, but enough, fortunately, to establish the correctness and synonymity of the names. Not more than two miles from East Malling is an orchard containing Hedelfingen, Early Rivers, Kent Bigarreau and Napoleon Bigarreau, the trees being perhaps 25 or 30 years old. Of the four varieties, Napoleon seems likely soon to be wiped out by Bacterial Canker ; Kent Bigarreau is considerably injured, Early Rivers very little, and Hedelfingen not at all.

Since the fruit of Hedelfingen is easily identified—there being, so far as the writer knows, no closely similar variety—it seems that in this case local conditions must have greatly modified its susceptibility. The other three varieties have behaved almost exactly as East Malling experience would have led one to expect. It is possible that the chief factor in the resistance of Hedelfingen is age of tree. It has frequently been stated that if trees can be carried on beyond a certain age they become resistant to Bacterial Canker, or at least to " gumming ". Whilst none of the trees at East Malling is old enough to provide evidence on this point, it seems from the experience of growers that it is seldom if ever true of Napoleon Bigarreau and some other varieties. But it may be true of Hedelfingen. The only alternative explanation would appear to be that local conditions—presumably soil or cultivation—can radically alter the relative susceptibility of varieties. Cherry growers in Kent have long believed that trees in grass, or, in fact, under any conditions that tend to reduce vigour of growth, are less susceptible to " gumming " than trees that are induced by cultivation or other means to grow rapidly. The opportunity to test this theory has not so far occurred at East Malling. It is possible that it may be more applicable to some varieties than to others.

A second case occurred on an isolated fruit farm in the south of England. A large area planted with standard sweet cherries had, within four or five years of planting, been very severely injured. The soil was somewhat variable, particularly in its depth ; but there did not seem to be any considerable areas where the trees were less severely affected.

Half a mile from the edge of this orchard, to the west, was a small area of bush cherries, of many varieties, including, it was understood, at least three of the varieties in the orchard. The trees came from a different source, and were planted a year later than the large orchard. The soil appeared similar, and equally variable. In the whole of this plantation not a single instance of infection with Bacterial Canker could be found.

For such a striking difference, perhaps, no single explanation could be adequate ; a variety of circumstances may have contributed to it. But since the trees in the large orchard were already dying in large numbers within four years of planting, and since no local sources of infection could be found—although the neighbouring woods contained many fine healthy wild cherries—it seems possible that the initial infection came with the trees, and that those in the small plantation were healthy when planted. Perhaps the fact that the small plantation was to the west of the large orchard, the prevailing winds being west and south-west, had saved it from infection up to the time of the writer's visit. There is some slight evidence which suggests that bacterial infection can travel considerable distances on the wind, even where no cherries or other host plants intervene.

A third case, referred to by Dr. Wormald on a subsequent page (p. 37), concerns Roundel. As the explanation here is likely to be concerned with incorrect nomenclature, the case is discussed under another heading below.

Degree of Susceptibility at East Malling.

The following classification of varieties according to their susceptibility to Bacterial Canker will at least serve as a rough guide. It is based entirely on field observations; very few have been confirmed by laboratory examination for the presence of bacteria. The symptoms of Bacterial Canker, however, are usually distinct enough to ensure at least a low proportion of error in observation. Since gumming in cherries, and even the death of branches or whole trees may be due to more than one cause—e.g. infection by Stereum or Verticillium—there may have been a very few mistakes. But it is most unlikely that mistaken observation could account for any but a few of the minor differences shown in the Table.

In the Table the groups are given in order of decreasing susceptibility. Group 1, "extreme", includes only varieties of which more than one tree has either been completely killed, or has had to be cut down below the crotch. Most of these varieties have been cut down or replanted more than once. One variety, referred to below, has been completely lost; several have been saved only by constant reworking in the nursery.

The varieties in Group 2, "considerable", have in all cases had one tree either killed or cut down below the crotch. In nearly every case the other tree or trees have been considerably but less severely injured.

The third group, "slight or moderate", includes those varieties which have at times lost a branch of at least considerable size, but have neither had to be repeatedly cut nor have lost whole trees.

In Group 4, "very slight", are placed those varieties which have never yet had a branch infection of any consequence. A few, as for instance, Newington Black, have at times had a considerable number of spurs infected and killed; others have sometimes been moderately or even severely affected by Bacterial Leaf Spot. But experience has shown that, as far as branch, crotch, or stem infections are concerned, none of these varieties has been seriously injured.

It is quite certain that longer experience will make it necessary to transfer some of the less susceptible varieties to a more susceptible category. At the end of 1935, for instance, Black Tartarian E and Waterloo would have been included in Group 4; in 1936, however, all three trees of Black Tartarian E, and one tree out of three of Waterloo lost branches of good size, clearly through Bacterial Canker; they were therefore transferred to Group 3. It may eventually be necessary to transfer some varieties to a less susceptible group; only long experience can show the need for this.

Table of Susceptibility.

1. Extreme.	2. Considerable.	3. Slight or moderate.	4. Very slight.
	SWEET CHERRIES.		
Abundance.	Black Downton.	Baumann's May A.	Bauman's May B.
Bedford Prolific B. (not Roundel).	Burr's Seedling.	Bedford Prolific A. (?=Roundel).	Big. de Jaboulay (=Early Lyons and Frühe Rote).
Beeve's Heart.	"Early Purple Gean."	Belle Agathe.	Black Cluster.
Belle de Droures.	Goodnestone Black.	Black Eagle.	Black Oliver.
Big. de Mezel.	Great Bigarreau.	Black Elton.	"Buttner's Rote" (White).
Big. de Schrecken.	Guigne très Précoce.	Black Tartarian E.	Caroon A.
Bing.	Hooker's Black.	California Advance.	Cleveland Bigarreau.
Bloor's Heart.	Hoskin.	Circassian.	Early Cluster.
Burbank.	Kent Bigarreau (=Amber Heart).	Cryall's Seedling.	Emperor Francis (1 tree).
Centennial.	Knight's Bigarreau.	Dunn "Mazzard".	Frogmore Bigarreau.
Chapman.	Ludwig's Bigarreau.	Early Amber.	Ham Green Black.
Early Bigarreau.	Norwegian.	Early May (=Mumford).	Hollander.
Florence.	Ord.	Early Rivers.	Kassin's Frühe.
G. d'Hedelfingen (=Bradbourne Black).	Pontiac.	Elton Heart.	Lambert.
"Grosse Schwarze Knorpelkirsche."	Ramon Oliva.	Gov. Wood.	Large Black "Mazzard".
Guigne d'Annonay.	Royal Queen.	Knight's Early Black.	"Longley's Black Eagle."
Ironsides (="Ohio Beauty").	St. Margaret's (=Noble).	Lulsley Early Black (?=Früheste der Mark).	Maiden's Blush.
Late Amber.	"Thamenkirsche ganz Süsse Schwarze."	"Noir de Schmidt" (White).	Newington Black (=Caroon B).
Lewelling.	Wellington A.	Old Black Heart A.	Noir de Guben.
West Midlands Bigarreau.		Old Black Heart B.	Norbury's Early Black.
Yates' Seedling.		Philpott's Favourite (=Big Reverchon).	Nutberry Black.
Yellow Spanish.		Rockport Bigarreau.	Perserving "Mazzard".
		"Schneider's Späte Knorpelskirsche" (early).	"Red Turk."
		Smoky Dunn.	Rodmersham Seedling.
		Strawberry Amber.	Roundel.
		Turkey Heart.	Small Black "Mazzard".
		Victoria Black.	Sutton's Purple.
		Wellington B.	White Heart.
		Windsor.	
	"DUKES."		
Planchoury.	"Belle de Franconville."	Archduke.	"Belle de Chatenay."
	May Duke.	Ronald's Late Duke.	Belle de Choisy.
	Reine Hortense.	"Rote Mai" (?=Ronald's Late Duke).	Empress Eugenie.
			Nouvelle Royale.
			Olivet.
			Royal Duke.
	ACID CHERRIES.		
None.	Flemish Red.	Morello A.	Carnation.
	Griotte de Portugal.		Coe's Carnation.
	Gros Gobet (=Montmorency Short Stalk).		Kentish Red (Self-fertile).
	Wye Morello.		Kentish Red (Self-sterile).
			Morello B (and others ?).
			Ostheimer Weichsel.
			Triaux.

Several varieties added to the collection more recently than those in the Table have already proved distinctly susceptible to the disease. These include August Heart, Beechcroft, Bowyer Heart, Dangler, Leicester, Napoleon Bigarreau, Peggy Rivers, Pointed Heart, Ronald's Heart, Sutton's Prolific (distinct from Sutton's Purple), Werder's Early Black, and two late White Cherries not yet identified. It is not unlikely that some of these will eventually prove to be less susceptible than they now seem.

Questions of Nomenclature.

Nearly all the names given in the Table are believed to be correct. Where two or more varieties are entered under one name—no alternative name for either having yet been found—they are distinguished by letters. Black Tartarian E is the variety distinguished in this way by Crane (1). No other "Black Tartarian" has yet been in the East Malling collection long enough to be included.

In a few cases the names given are clearly wrong; these are placed in quotation marks. The variety received as "Buttner's Rote" (properly a black cherry), is white, as is also the variety received as "Noir de Schmidt". Until these varieties can be identified, it seems best to continue using the incorrect names.

The synonyms given (except those queried) have been confirmed.

A case of apparent difference in susceptibility of a variety under different conditions, mentioned by Dr. Wormald on a subsequent page (p. 37) of this Number, probably arose through incorrect nomenclature. "Roundel" was there included amongst the more susceptible varieties. At East Malling, and in the experience of several growers, Roundel is found to be one of the most resistant. In all probability, two different varieties are here concerned. There is a group of varieties, including apparently at least two called "Bedford Prolific" (or "Bedford Black"), which are closely similar to Roundel in every way. In 1923 East Malling received buds of "Roundel" and "Bedford Prolific" which were supposed to be identical; and in 1924, from another source, a tree of "Bedford Prolific". Of these, "Roundel" has grown fairly well, has always been healthy, and has been bearing increasing crops for some years. In tree and fruit characters it seems identical with the Roundel commonly grown. The first "Bedford Prolific" ("B") proved so highly susceptible to Bacterial Canker that both trees were killed in spite of efforts to save them, and the variety was lost to the collection. The second "Bedford Prolific" ("A") is in many ways very similar to Roundel, but appears to differ in producing a slightly more spreading tree; its blossoming season is consistently somewhat earlier, and it appears rather less resistant to Bacterial Canker than Roundel. Except for these differences, it could so far easily be mistaken for Roundel.

The susceptible "Roundel" mentioned by Dr. Wormald may therefore have been one of these "Bedford Prolifics".

Various Types of Bacterial Infection of Cherries.

The attempt to control Bacterial Canker by cutting out infections has yielded a mass of observations on the susceptibility of varieties to various types of infection.

From the grower's or practical point of view, the types of bacterial infection most commonly met with are: Leaf spot, bud infections on shoots of the previous summer (presumably through the petiole scar), spur infections, together with infections of the bark and cambium of branch, crotch and stem, leading usually to cankers. Varieties differ not only in their susceptibility to infection in general, but also in the relative degree to which they are subject to these various types of infection. It has been observed for several years, for instance, that of two varieties in the East Malling cherry rootstock trials, Early Rivers is rather highly susceptible to leaf spot and little to canker, whilst Bigarreau de Schrecken is comparatively resistant to leaf spot but susceptible to canker. The first of these observations confirms that recorded by Dr. Wormald on a subsequent page (p. 41).

Whilst this observation on Early Rivers has frequently been confirmed, certain other observations of peculiar types of infection occurring chiefly on one or two varieties are more occasional, and are not always confirmed by subsequent experience. Thus, in the spring of 1930, it was observed that the two trees of "Bedford Prolific B" had most of their flowering spurs killed, apparently by bacteria, but were otherwise little injured. This observation was not repeated; in 1932 both trees developed stem or crotch infections, which spread so rapidly that, in spite of repeated cutting back, both trees died in 1933, without providing scions for further propagation.

In the spring of 1931 a peculiar type of spur infection not previously seen was noticed on the two trees of the variety known in Kent (probably incorrectly) as "Ohio Beauty". On many spurs certain flower buds failed to grow, whilst other buds produced only small and poorly formed flowers; most of these spurs were completely killed before any fruit developed. Shortly afterwards the same type of infection was found on the two trees of the Herefordshire variety "Ironsides"; it was not found in that year on any other variety. This was the first clue to the identity of these two varieties, subsequently confirmed by fruit and stone descriptions.

In the summer of 1935 several varieties developed many spur infections, but were otherwise almost uninjured, except in some cases by leaf spot. Of these the most interesting was "Newington Black", a name usually given as a synonym for Caroon. Both of the trees of this variety had many spurs killed,

whilst those of the variety received as "Caroon A", close by, were both practically uninjured. Careful observation of the fruit in 1936 made it seem very probable that the two varieties, though closely similar, are not identical. It may be remarked here that both these varieties, received from the Sittingbourne district, are much later in ripening that the "Caroon C" found in old orchards near East Malling. Though Hogg (2) and Bunyard (3) disagree in certain particulars, they agree in describing Corone (Caroon is regarded as a local name) as late ripening.

A type of infection observed in certain seasons in low-worked nursery trees of Early Rivers (rarely in older trees and those of other varieties), is, in the writer's experience, unique amongst fruit tree diseases caused by micro-organisms. It seems quite clear that considerable areas of bark on the tree stem may be invaded by the bacteria, even though the cambium is practically untouched. Such infections do not appreciably injure the tree, but they make it, in bad cases, so unsightly that it could not be sold. A moderate proportion of the Early Rivers trees raised for the East Malling rootstock trials had this type of infection in 1927. Some of the less unsightly specimens were planted in the trial; they soon developed smooth stems showing no sign of the injury. This type of infection has not so far been specially examined for the presence of bacteria; but the writer is in no doubt that the cause is the same as that of the very large number of bacterial infections he has examined.

Still another type of infection has been found, chiefly in Morello. The variety grown as Morello ("A") in the rootstock trials came from a different source, and appears in several respects slightly different from the Morello ("B") in the variety collection. Of the former, two or three trees out of more than two hundred have been lost, apparently through Bacterial Canker; but the variety is generally resistant, except to one type of infection. In cutting out infections of Brown Rot, the writer has frequently found dead or dying twigs in which Brown Rot had clearly nothing to do with the injury. In practically every case there was a small infection, girdling the twig, at the tip of one year's growth and the base of the next. Such infections, mostly found in May and June, were usually at the base of the shoot formed two years previously. The twigs at the point of infection were rarely more than 6 to 8 mm. in diameter, and often much less; the injury to the tree was therefore slight.

Rootstock Influence on Susceptibility.

In view of the fact (4) that certain apple rootstocks have appeared to influence the susceptibility of the scions worked on them to various fungus diseases, it is at least possible that cherry rootstocks might influence the susceptibility of the scion to Bacterial Canker.

Observation, however, strongly suggests that any influence there may be is not direct, due in some way to the union of resistant with susceptible tissues, but indirect. If it can be shown to exist, it will probably be associated with an influence of the rootstock on the vigour of the scion, presumably through some nutritional factor.

Since nearly all the sweet cherries so far planted at East Malling have been "low-worked", with stems of the scion variety, the union occurs practically at ground level, and does not provide a good opportunity for observing the possible extension of a bacterial infection into the rootstock. The large young orchard already mentioned as having been very severely injured by Bacterial Canker, however, consisted entirely of "high-worked" trees, the stem being formed by the stock. Here some interesting observations were made on the relative susceptibility of stock and scion.

Whilst some of the stocks were much damaged by the disease, several were clearly very resistant, and in these cases the scion was as clearly highly susceptible. The bacteria had invaded apparently every cell of the scion tissue, but seemed to have been completely unable to invade the stock tissue. The resistant stock tissue did not appear to have made even the small amount of scion tissue in direct union with it any more resistant than it normally would have been. There does not therefore seem to be much hope of increasing the resistance of susceptible varieties by either low or high working on the stems of resistant stocks. These stocks may, however, be useful for "frame-working" susceptible varieties; they could not be expected to prevent the death of certain branches down to the point of union, but they should prevent crotch infection, and the subsequent loss of the whole tree.

SUMMARY.

Observations on the East Malling collection of cherry varieties have shown that:—

1. Varieties differ widely in their susceptibility to Bacterial Canker.

2. There are several types of infection, of leaf, spur, branch and stem, and varieties differ in their relative susceptibility to these various types.

3. Whilst rootstocks may indirectly influence the susceptibility of the scion, they do not appear to influence it directly; susceptible scions on resistant rootstocks may be completely killed to the point of union, though the stock is entirely uninjured.

4. The reliability of the observations, and certain questions of nomenclature, are discussed, as is also the possibility that local conditions may influence the relative susceptibility of varieties.

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BACTERIOSIS OF STONE FRUIT TREES IN BRITAIN

VI. FIELD OBSERVATIONS ON BACTERIOSIS OF SWEET CHERRY TREES

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General Observations.

For many years there have been serious losses in cherry orchards in the south-eastern and western fruit-growing counties of England, young trees dying completely and old established trees losing large limbs. The cause of this so-called " die-back " was long disputed, but during recent years evidence has accumulated which shows that much of it is a result of bacterial infection, and, in fact, that is has much in common with a similar disease of plum trees in which one form of die-back is now known as Bacterial Canker because it has been found to be due to bacterial lesions on branches and stems (18, 19). Similar bacterial lesions are associated with die-back in cherry trees although in cherries the exudation of gum from such lesions is usually far more profuse than in plum trees. Gummosis itself has been attributed to various unfavourable factors ; it would appear that any condition which arrests the downward flow of elaborated food material will induce gummosis in cherry trees, and one of the most potent of these factors is bacterial infection which produces cankers on branches and stems.

The association of bacteria with die-back in cherries in Britain first came to the writer's notice as long ago as 1919 when his attention was drawn to the condition of a cherry orchard at Ham Street, near Ashford, Kent. On visiting the orchard, in June of that year, trees of Black Tartarian were seen with many dead branches, some of which had been dead for some time while others were just beginning to show evidence of disease by yellowness of foliage and wilting of leaves and fruit. The bark of the affected branches, when cut, was seen to be quite brown, and a microscopic examination of the cortical tissues towards the lower extremities of the lesions showed dense bacterial masses. Further back the xylem also was discoloured and fungal hyphae were present in the vessels. As bacteria only were found in tissues bordering on the healthy parts it seemed likely that they were the primary cause of the damage and that the fungus in the xylem was secondary.

In May 1923, a similar condition was seen on a fruit farm in East Sussex. In this case die-back was accompanied by severe spotting of the leaves, and

the spots when teased out in water yielded bacterial masses, thus suggesting that there might be some connection between the leaf spotting and die-back. One orchard consisted of four varieties, Cluster Black Heart, Early Rivers, Waterloo and Black Tartarian, all affected to a greater or less extent. The Cluster Black Heart was worst, only a few trees appearing quite healthy ; many had branches killed back almost to the crotch and some trees had been headed back, because of the disease, and regrafted. At least half the trees of this variety were seriously affected and had one or more dead branches, while others showed leaf spots only. The typical appearance was for a branch to have a dead terminal portion (one-year-old) bare of leaves ; lower down the young shoots bore some leaves which were spotted and others completely blackened. The spots eventually fell out leaving " shot-holes ". There was evidence that some of the flowers had been attacked, for they were withering, their pedicels being wholly or partly brown and containing bacteria. Certain Early Rivers trees were also seriously affected but, on the whole, this variety suffered less than Cluster Black Heart. On Waterloo and Black Tartarian the disease was less severe.

In another orchard on the same farm a few young trees of Black Eagle were affected. The spots on the leaves of this variety were reddish brown, circular, usually with a lighter translucent centre, and up to about 1·5 mm. in diameter.

On Black Tartarian leaves the spots were generally larger (up to 3 mm. in diameter) and more scattered ; " shot-holes " were very numerous in some leaves.

Another visit to the Ham Street orchard, in 1923, showed that here, too, the dying back of the branches of the Black Tartarian trees was accompanied by bacterial leaf-spotting, thus offering further evidence of the connection between the two symptoms. The owner said that, until that year, there had been little die-back since the outbreak in 1919. The abnormally cold damp weather of 1923 was probably responsible for the severe leaf infection in that year. Spotted leaves, often with withered margins, were common. Other varieties in the same orchard were Early Rivers, Napoleon and Turk, and these were apparently free from the disease, though observations in other localities showed that these varieties also are susceptible.

In the same year (1923) bacterial leaf spot of cherries was seen in several localities, again usually associated with die-back. In an orchard of three-year-old trees at Bramling, near Canterbury, it was accompanied by serious dying back of the branches, many trees having a " stag's head " appearance because of the dead leafless branches ; others were dead down to the crotch and in such cases the whole scion head of a tree was killed, the stock-stem only being alive. One tree had a lesion two feet long on the " Gaskin " stock-stem ; it had yellowish foliage and was evidently dying. Fungal fructifications of a

species of *Cytospora* were found on the discoloured bark ; towards the extremities of the lesion, however, no mycelium could be found, but numerous bacterial rods in dense masses were present. Most of the trees showed severe leaf spotting and on some of them, the spots were not only on the foliage of the scion head but also on the leaves of the " feathers " on the Gaskin stem.

This orchard was visited during the following year and the disease again found to be rife. It was ascertained that the orchard consisted originally of three hundred and twenty trees, planted on the site of an old hop garden in the winter of 1920-21. In 1922 about twenty had shown the disease, so these had been grubbed and replaced by others, but in 1923 it was found necessary to grub forty more. Most of the trees remaining in 1924 showed at least a trace of the disease. A few with small yellowish leaves were dying and had stem lesions, but most of the trees showed a dying back of the branches, the terminal portion of one or more branches being dead and bare of leaves, while the lower parts, below the lesions, bore spotted, but otherwise normal, leaves. Of the varieties grown, Roundel Heart* was the first to succumb and the gaps had been planted up with Bigarreau de Schrecken which also became attacked. The disease was severe on Black Eagle, less pronounced on Waterloo, whilst Early Rivers, Turk and Amber Heart were only slightly affected. Again it was found that although fungal hyphae were present in the diseased parts of stems and branches, the tissues bordering on the healthy parts contained bacteria only which oozed out in dense masses on teasing out portions of the cortex in water.

More recent observations have been made on the variety trial plots of cherries at the East Malling Research Station where certain varieties have proved to be very susceptible to bacterial infection.† All parts of the trees except the roots may be attacked, for lesions have been found on stems, branches, shoots, buds, leaves, flowers and fruit. These different forms of damage have come under examination and organisms associated with the various types of lesions have been isolated.

Symptoms of Bacteriosis in Sweet Cherry Trees in Britain.

Stem and Branch Lesions.

As in plum trees, the bacterial infection on stems and branches of cherry trees induces necrosis of the cortical and phloem cells, accompanied by

* In this plantation the variety known as Roundel Heart was apparently very susceptible. In an orchard at Wye (see p. 41) Roundel Heart showed less infection than most of the other varieties, and this relatively low susceptibility has been observed at East Malling also. In the nomenclature of cherries there are many anomalies and it is possible that the variety at Bramling was not the same as that grown at Wye under the same name.

† The field observations at East Malling have been carried out in collaboration with Mr. N. H. Grubb whose account (8) of the relative susceptibility of the varieties examined will be found on another page (p. 25).

gummosis. In cherries the gum is usually very copious, and in wet weather it oozes out to form translucent masses on stem, crotch or branches. The cambium is destroyed so that, when a lesion does not completely girdle the stem or branch, development is arrested over the affected area and the continued growth of the parts not attacked results in the infected region remaining sunken below the general level. As a rule a lesion does not increase in size after June, and if a branch is not girdled by that time the unattacked tissues round the margin of the lesion develop callus which tends to cover up the cankered area. Such cankers may cause little or no permanent injury.

During July and August, therefore, there is usually a border of callus to such a lesion, the bark cracks and a canker results. In these cankers the organism dies out, and attempts at isolation during July and August may fail. It has been observed, however, that some lesions do not produce callus so early and the organism has been isolated from cankers in late July and early August. Such lesions were observed during the rather cold and wet summer of 1936, and it would seem that these conditions prolong the development of the bacteria in cankers during summer.

A girdling canker, destroying the cortex, phloem and cambium, but not at the same time the xylem, may not check the ascent of sap while arresting the downward flow ; the branch thus becomes " ringed " and the accumulation of foodstuffs immediately above the lesion induces increased development of the tissues in that region so that just above the canker the branch is abnormally thickened (Fig. 6). Eventually, however, on the parts terminal to a girdling canker, the foliage turns pale green or yellowish, tends to be " curled ", and finally withers. The final result therefore of a girdling canker is to kill those portions above it.

On a maiden tree the infected region on the stem is indicated by failure of the buds to develop there. Above the lesion the tree may come into leaf but the leaves turn yellowish, are narrow and curled, and eventually wither (Figs. 4 and 5). Below the canker the buds are stimulated to increased development and they grow out to form strong healthy shoots, often, however, with spotted leaves as a result of infection by bacteria splashed from the canker.

Bud and Spur Infection.

Isolated buds are often killed and one-year-old twigs may be seen with one or more buds failing to develop while the rest of them grow out normally and come into leaf. In common with other lesions on infected trees these dead buds contain numerous bacteria. There is usually a lesion round the base of a dead bud and if this is extensive it may girdle the twig. The dying back of one-year-twigs as a result of these nodal infections is a common

occurrence, particularly on young trees, and on maiden trees the "leaders" may be killed back in consequence.

The buds on the spurs of older branches may also become infected. One or two buds only on a spur may be killed but often the axis of the spur is invaded and all the buds are destroyed. Again, such a lesion may extend into the branch itself, producing a canker which may girdle and kill the branch. Similarly on two-year-old trees, the main stems become infected from the spurs.

Some varieties appear to be more susceptible to spur infection than any other form of bacterial attack. In 1935 at East Malling several varieties had many spurs killed but no other serious damage; on some of the trees even leaf spot infection was slight.

Bacterial Shoot Wilt.

Blackened lesions sometimes appear on the young green shoots. As they are usually much longer than broad they may extend for several centimetres along one side without actually girdling. Such a lesion causes a distortion of the infected shoot which tends to curve over towards the side bearing the lesion, but frequently shoots are girdled and the terminal leaves wither. Bacterial shoot wilt is almost invariably accompanied by severe leaf spotting.

Similar lesions have also been observed on shoots in nursery stool beds, but bacterial shoot wilt in stools and layer rows appears to be rare, although wilting due to other causes occurs.

Leaf Infections.

Bacterial infection of the foliage usually takes the form of leaf spots. When these are numerous and crowded they are very small and angular; when more scattered they are larger, 1·5 to 3 mm. in diameter, and circular. The spots at first have a water-soaked appearance, but later the dead tissues turn brown and drop out leaving "shot-holes". If the tissues of a young spot are teased out in water, bacteria ooze out in a dense gelatinous mass which may be seen under fairly low power of the microscope (2/3″ objective, and eyepiece ×6), although higher magnification is necessary to show the bacterial rods. If young spots are examined in very wet weather some of the bacteria may be seen to be actively motile; usually, however, the only movements they show are the passive oozing out from the ruptured cells and "Brownian" motion.

When the spots are numerous they sometimes coalesce to form irregular necrotic blotches causing the leaves to become distorted. Often the spots are crowded towards the drooping tips of the leaves, suggesting that the accumulation of rain water at the tips brings the bacteria to this part of the leaf and encourages infection. What appears to be infection through the marginal

" water pores " (hydathodes) has been observed, and the withering of the leaf margins sometimes seen appears to be an extension of such infection from the " teeth " into the adjoining tissues.

Infection of petioles and midribs also occasionally occurs. This is a result of the extension of the bacteriosis from an infected spur upwards along the petioles into the laminae ; from the midrib it may then extend into the larger veins. Infection of the leaves in this way is unusual, however, and has been seen only in very wet weather in early spring before the leaves have fully expanded. When there is much infection about the time when the buds are expanding or soon after, the young leaves may be killed before they fully expand.

Leaf infection is usually severe in the neighbourhood of branch lesions, particularly on leaves borne on spurs immediately below the cankers ; this suggests that the bacteria are carried down by rain from the cankers on to the foliage beneath.

Infection of Flowers.

Occasionally wilted flowers are found with bacteria within the tissues. Whether flowers may become infected directly from without, or whether they are only invaded from the spur on which they are borne has not been ascertained. This condition is rarely seen on sweet cherries.

On acid cherries there is a more serious blossom blight which, from the presence of numerous bacteria within the discoloured organs, appears to be a bacterial disease ; the organism usually isolated from these lesions is different, however, from both of those causing bacteriosis of sweet cherries in Britain, but its identity has not yet been determined.

Fruit Infection.

Lesions containing bacteria have been seen on the young green fruit, but this condition, too, is rare. It has been found on cherries in three localities but only on a few fruits, so that there is no reason to suppose that this form of infection is likely to cause any serious loss.

Varietal Susceptibility.

Observations in commercial plantations, as recorded above, show that certain varieties of cherry commonly cultivated in Britain are very susceptible to bacteriosis. Among the most susceptible would appear to be Black Tartarian, Cluster Black Heart, Bigarreau de Schrecken, Black Eagle and (as shown below) Bradbourne Black, while Waterloo, Turk, Amber Heart and Early Rivers are also sometimes seriously attacked.

Detailed records taken in an orchard at Wye, near Ashford, Kent, during the summer of 1936, provided information on the relative susceptibility of

certain cherry varieties to bacterial infection. Over three hundred and fifty trees, including nine varieties,* were examined individually and the degree and type of infection noted. The number of trees cankered in each variety was recorded, and the degree of leaf spotting was determined by using the numbers 0 to 4, 0 indicating no infection, and 4 very severe spotting involving most of the leaves.

The varieties, and their order in increasing degree of infection with regard to cankering (stem and branches) and leaf spot, are here shown.

Cankering.	*Leaf Spotting.*
Frogmore.	Frogmore.
Governor Wood.	Governor Wood.
Roundel Heart.	Roundel Heart.
Early Rivers.	Amber Bigarreau.
Napoleon.	Emperor Francis.
Waterloo.	Napoleon.
Amber Bigarreau.	Waterloo.
Bradbourne Black.	Bradbourne Black.
Emperor Francis.	Early Rivers.

Frogmore proved to be the most resistant to both cankering and leaf spotting. On the thirty-four trees of this variety there was only one tree with canker—a small stem lesion causing no appreciable damage—and the trees had very little leaf spot, thirty of them showing no leaf infection, and the other four only a trace.

At the other end of the list is Emperor Francis of which seven trees were cankered out of twenty-seven or 26 per cent. ; Bradbourne Black proved also very susceptible, with very severe leaf spotting and 25 per cent. of the trees cankered. Leaf spotting was particularly bad on Early Rivers, a variety which is evidently very susceptible to this form of infection, although cankering was not so severe as on most of the other varieties in this orchard (three trees cankered out of forty-three).†

On the whole it will be seen that if we except Early Rivers there is a fairly close relationship between susceptibility to leaf spot and degree of damage by cankers. This is particularly noticeable in the varieties Frogmore and Governor Wood, which appear at the head of both columns as showing little infection, and, at the other extreme, in Bradbourne Black which is very susceptible to both forms of infection.

* The writer is greatly indebted to Mr. W. G. Kent and Mr. C. R. Thompson for their help in taking these records.

† At East Malling also, Early Rivers has proved to be very susceptible to leaf spotting. Of three varieties (Early Rivers, Turk, and Bigarreau de Schrecken) grown on one plot here, Rivers is the least affected by canker and most by leaf spot. Rivers is generally regarded by growers as relatively resistant to the canker form of infection.

With regard to the shoot wilt form of bacteriosis the greatest number of affected shoots were found on Napoleon and Waterloo, fewer on Amber Bigarreau, Bradbourne Black and Roundel Heart.

Organisms causing Bacteriosis of Cherries in Britain.

Many isolations have been made from the stems, branches, buds and leaf spots, of infected trees at East Malling and in other localities, and inoculation experiments on stems, branches and leaves with these organisms have shown them to be pathogenic. An account of these experiments will be published later, but it may be stated here that the bacteria isolated fall into two groups, one conforming to *Pseudomonas prunicola*, which has been shown to be the cause of a shoot wilt of plum trees at East Malling, the other to *P. mors-prunorum* which produces Bacterial Canker in plum trees. Each of these organisms has been isolated from stem and branch lesions and from leaf spots on sweet cherries.

P. prunicola has been obtained several times from lesions on trees at the East Malling Research Station, once also from a branch lesion on a commercial fruit farm at East Malling and once from spots on fruit obtained from North Kent, while *P. mors-prunorum* has been isolated many times from specimens collected at East Malling and a number of other localities. It would appear then that *P. mors-prunorum* is the more generally distributed of the two organisms in Britain. On the other hand *P. prunicola*, or a form which appears to be indistinguishable from it, occurs in America ; moreover it is closely related to and perhaps identical with the lilac blight organism *P. Syringae*, which has been found on the Continent, in North America and also in this country. Reference to the work by American investigators who are studying the relationship of these organisms is given below.

On acid cherry trees at East Malling bacteria have been found associated with lesions on branches, with leaf spots, and in withered flowering spurs. The organisms isolated from these lesions are under investigation. One of them, from branch cankers, appears to be identical with *P. mors-prunorum*, but a detailed comparison has not yet been made ; another, isolated from leaf spots and blighted flowering spurs, is different from both *P. prunicola* and *P. mors-prunorum*, as well as from *P. Pruni*, found by Dunegan to cause leaf spotting of Morello cherries in America, but its identity has not yet been determined.

Bacteriosis of Cherry Trees in Other Countries.

In 1902 Brzezinski (4) found bacteria associated with cankering and gummosis in stone fruit trees in Poland, and concluded that in peaches, plums and apricots gummosis was caused by an orange yellow organism while in cherries it was an organism of which he stated, " ses colonies, obtenues en culture, sont blanchêtres, fortement opalescentes et particulières par leur structure ", but he offered no further description of the organisms.

In Germany, in 1905, there was an epidemic outbreak of gummosis and high mortality in cherry trees. The disease was investigated by Aderhold and Ruhland (1, 2) who isolated a bacterium from the lesions and succeeded in reproducing the disease by inoculations with this organism, which they named *Bacillus spongiosus*. The symptoms of the disease as affecting stems and branches agree with those observed in this country, but there is no mention of leaf infection ; hence the leaf spot phase if present was either not observed or, if noticed, was not considered to have any connection with the stem and branch lesions.

In the western States of North America bacterial gummosis is a serious disease in cherries, and it has come under the notice of several investigators. Griffin (7) was the first to show that this condition in Oregon was caused by bacteria ; he wrote, " It is in the more humid portion of western Oregon that its prevalence and destructiveness gives it the rank of a major disease." He isolated and described the organism, which he named *Pseudomonas cerasus*, and by inoculation experiments proved it to be the cause of the disease.

These results were confirmed by Barss (3) who gave a detailed description of the disease. His account of the cankers, the " blighting of buds and spurs ", and leaf infection, shows that the symptoms he observed are similar to those found in Britain.

The bacteria causing gummosis and sour-sap of stone fruit trees in California have been closely studied by E. E. Wilson (13, 14) who finds that there are two nearly related organisms which may cause these diseases. One is probably that described by Griffin, *Pseudomonas Cerasi*,* an organism which produces a green coloration in beef extract media, and a white organism which Wilson finds is similar to *P. prunicola*, described by the present writer as causing a wilt of plum shoots in Britain (17). By reason of the close affinity of the two organisms Wilson prefers to consider *P. prunicola* as a variety of *P. Cerasi* and names it *P. Cerasi* var. *prunicola*.

Other organisms have been found to cause bacteriosis of cherries in North America. Sackett (11) described a disease of the Wragg Cherry, and the bacterium causing it he named *P. Cerasi Wraggi*. More recently Dunegan (6) has described a leaf spot of Morello cherries which he finds is caused by *Bacterium* (*Pseudomonas*) *Pruni*, an organism which had previously been shown to cause serious leaf spotting of plums and peaches in North America.

C. O. Smith (12) has called attention to the close relationship not only between *P. prunicola* and *P. Cerasi* but also between these and *P. Syringae* (the cause of bacterial blight in lilac) and *P. citriputeale* (the citrus blast organism) ; it is suggested that all these bacteria may be identical.

* Wilson points out that " The possessive *cerasi*, is preferable to *cerasus*", hence the change in the last syllable of the specific epithet.

Rosen and Bleecher (10) in a study of pathogenic fluorescent bacteria, including a "pear blast" organism, conclude that "*P. prunicola* can hardly be distinguished from *P. Syringae* and from the pear blast pathogene either in pathogenicity tests or in cultural reactions, and the serological tests further emphasize its relationship to this group"; they state further that these three organisms "produce symptoms on pear twigs and leaves that are indistinguishable". These conclusions have recently been confirmed by Wilson (15). The pear disease described by these workers has features in common with a bacterial blossom wilt of pear trees in Britain at present under investigation at East Malling; this appears to be caused by an organism which in culture is found to have affinity with *P. prunicola* and may prove to be identical with it. The organism was isolated in 1929 from blighted flowering spurs (20) and again, in 1936, not only from spurs but also from fruit spots on pears.

In the hands of various investigators it has been found that, by artificial inoculation, *P. Syringae* can be induced to infect a number of host plants, and recently Rosen (9) has described "a disease of roses involving receptacles, calyx lobes, pedicels and petioles" caused by *P. Syringae*. This organism has been found on lilac in Britain (21) and a similar "blight" of *Forsythia* in this country is probably caused by the same bacterium. If it is eventually found that *P. prunicola* and *P. Syringae* are one and the same organism and can infect all these host plants under field conditions the possibility of inter-infection between ornamental bushes and various species of fruit trees is a point of practical importance not only in nurseries where these host plants may be growing in close proximity but also in mixed fruit plantations.

Comparison of Cherry Bacteriosis with that of other Species of Prunus.

The various symptoms of bacteriosis in cherry trees have much in common with those in plum trees. Cankers on stems and branches, shoot wilt, leaf spots and fruit spots are found on both hosts. As already mentioned, gummosis is a conspicuous feature of bacterial cankers in cherries. In plums the exudation of gum on the surface of the lesions is infrequent and only in one variety, Bradley's King damson, has gummosis been found at all comparable with that in cherries. Again, in cherries, the cankering of branches is more common than in plums; the dying back of individual branches is exceptional in plum trees, even in plantations where stem cankers are numerous.

Foliage infection is more serious, on the whole, in cherries than in plums; marginal scorching and complete withering of leaves, as described above for cherries, has not been observed in plums.

The sequence of infection in plums and cherries is similar. In both, the stem lesions develop during autumn, winter and early spring; the organisms in such lesions die during the summer; but leaf and shoot infections occur in

spring and summer and the leaf spots are probably the sources of infection for stems and branches in the autumn.

In both plum and cherry trees in Britain the organism most generally associated with infections is *P. mors-prunorum* which has been isolated from lesions on stems, branches, shoots, leaf spots and fruit spots on both hosts. In cherries *P. prunicola* also has been isolated from lesions on all these organs, while in plums it has been isolated from green shoots but not yet from cankers on the woody branches and stems.

Again, organisms which in their cultural characters conform to *P. mors-prunorum* rather than to *P. prunicola* have been isolated from peach (stem), myrobolan or cherry plum (stem, branch, leaf), Morello cherry (branch), almond (stem, shoot, leaf) and *Prunus Pissardii* (stem).

A Consideration of Control Measures.

Bacterial diseases of trees, as instanced by Fire-blight of pear and apple trees in America and elsewhere, and walnut Blight, are notoriously difficult to control. Bacteriosis in stone fruit trees is also proving a serious problem, although from a knowledge of the habits of the organisms in the field, methods of control are being sought along several lines.

In the first place mechanical injuries such as pruning and pinching back should be avoided as far as possible during autumn, winter and early spring when stems and branches are more susceptible to infection than in summer. Lesions on stems and branches are inconspicuous during winter and so are difficult of detection. They do not become noticeable until the trees resume growth in spring, and it may then be found necessary to cut out diseased parts, not only to prevent possible further extension of the cankers, but also to remove tissues containing bacteria which may be carried by rain on to the opening leaves to cause leaf spots. This excision of infected parts should be carried out early in the year, as soon as the lesions can be detected ; if left until later the operation is useless for, as already mentioned, the organisms in the cankers die out during the summer and most cankers which have not girdled by mid-summer tend to become healed without treatment. Any open wounds left by cutting out infected parts should be covered with an antiseptic dressing. In America good results are reported against Fire-blight by dressing the cuts and sterilizing the tools with a mercury-glycerine disinfectant, while a zinc chloride preparation proved effective when applied to cankers without excision (5). Whether such treatment would be effective against bacteriosis in cherries is not known but it merits trial.

From field observations and results of inoculation experiments it is assumed that the branches and stems become infected in autumn, and the leaves in spring and summer. Attempts to control the disease by spraying have been directed

therefore to applications to branches and stems in autumn. Bordeaux mixture has been recommended for trial, for copper sulphate has been found to be toxic to the organisms at very low concentrations. Certain growers have reported that they have obtained some measure of success in this way, although controlled spraying trials have not yet given satisfactory results. If Bordeaux mixture is applied for this purpose care should be exercised to coat thoroughly branches, crotch and stem with the spray.

Spraying the foliage has given some degree of control for the leaf spot phase, but certain varieties of cherries are subject to spray injury from Bordeaux mixture and the method cannot be recommended for general use. Thus, in certain spraying trials at the East Malling Research Station, Bordeaux mixture caused a bronzing of the foliage and early leaf fall on the varieties Napoleon, Early Rivers and Bigarreau de Schrecken, while Frogmore was more resistant to the treatment.*

"Framework grafting", using resistant varieties for the stems and main branches, is being investigated as a possible means of controlling Bacterial Canker in plum trees. There is hope that for cherry trees, too, resistant stocks will be found on which the more valuable, but more susceptible varieties may be worked. In a plantation in Sussex it was seen that on certain trees, high-worked on seedling stocks, the scions were killed right down to the union, with no extension of infection into the stocks, while on other trees the lesions had extended from the scions into the stocks. This suggested that stocks may vary in susceptibility as much as, or possibly more than, scion varieties. At East Malling a start has been made in raising resistant stems for frameworking the more susceptible varieties of cherry, using one of the Devonshire Mazzards which appear to be the most resistant group in the variety collection.

It has been suggested that the application of lime to the soil might make trees more resistant to Bacterial Canker and (in cherries) the consequent gummosis. Experiments with cherries to test the value of liming for this disease have not yet been attempted, but in certain trials with young plum trees it was found that on plots receiving dressings of lime stem cankers resulting from inoculation with *Pseudomonas mors-prunorum* were, on the whole, greater than on plots not receiving lime. It is to be noted in this connection that some of the outbreaks of Bacterial Canker in Kent are in plantations on or near the chalk Downs. Moreover, Mr. N. H. Grubb informs the writer that, in 1926, he saw, in the Wyre Forest, near Bewdley, Worcestershire, fine healthy cherry orchards on very acid soil, where bracken is abundant and heather occurs commonly. It would seem doubtful, therefore, whether control of Bacterial Canker is to be obtained by applying lime to the soil.

* Observations by Mr. M. H. Moore.

ACKNOWLEDGMENTS.

The writer is indebted to Professor Salmon, of the South-Eastern Agricultural College, for his collaboration in the earlier observations recorded in this paper, and to Mr. W. G. Kent and Mr. C. R. Thompson, of the Kent Advisory Service, for their help in more recent years. He is grateful also to Mr. N. H. Grubb for continual interest and aid during the course of the investigation of these diseases at East Malling.

SUMMARY.

1. The serious damage caused by bacterial infection in a number of commercial cherry orchards is described.

2. The symptoms of bacteriosis in sweet cherry trees are, stem, crotch and branch cankers, shoot wilt, bud and spur blight, leaf spots, and, occasionally, fruit spots.

3. Certain varieties such as Bigarreau de Schrecken, Cluster Black Heart, Black Tartarian, Emperor Francis, Bradbourne Black and Black Eagle, have proved to be very commonly attacked. Frogmore and Governor Wood, in the observations recorded, have suffered less than other varieties.

4. The organisms causing bacteriosis in sweet cherry trees in Britain are *Pseudomonas prunicola* and *P. mors-prunorum*.

5. A summary is given of the work on bacterial diseases of cherry trees abroad.

6. Cherry bacteriosis is compared with that on other species of *Prunus*.

7. Methods for controlling these bacterial diseases in cherry trees are discussed.

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Fig. 1.
Healthy cherry tree (4 years old) as the above in Fig. 2.

Fig. 2.
Cherry tree (three years from planting) with Bacterial Canker on the stem.

Fig. 3.
Cherry tree (three years from planting) with branch lesions.

FIG. 4.
A young cherry tree with lesion on stem. Note the dead buds on the lesion and the curled leaves above it, as compared with the flattened leaves below.

FIG. 5.
The tree shown in Fig. 4 as seen a few weeks later. Above the canker the leaves have wilted; below, the buds have grown out to form strong shoots.

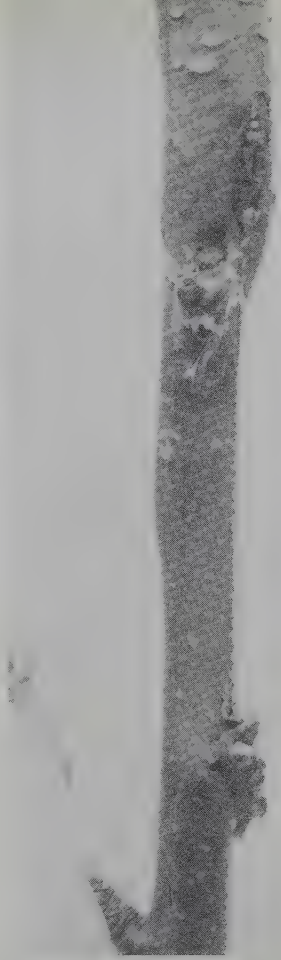

FIG. 6.
Portion of a cherry branch with a bacterial lesion. Immediately above the lesion the branch is abnormally thickened.

FIG. 7.
Nodal infection through buds.

FIG. 8.
Spur infections through buds.

FIG. 9.
Bacterial lesion on shoot, killing terminal portion.

FIG. 10.

Severe leaf infection causing withering of foliage; var. Cluster Black Heart.

FIG. 11.

Leaf infection: rather large spots more or less isolated; var. Black Tartarian.

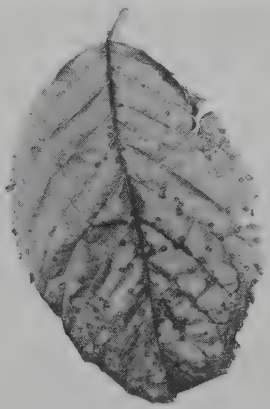

FIG. 12.

Leaf with small crowded spots; var. Black Eagle.

FIG. 13.

Young cherry leaves showing infection extending from petiole into the midrib.

OBSERVATIONS ON THE EFFECT OF POTASH SUPPLY ON THE WATER RELATIONS OF APPLE TREES

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In the absence of an adequate supply of potash the leaves of apple trees and of many other plants develop symptoms usually associated with a deficient water supply (1, 4). It appears that an abundant supply of potash is necessary for the maintenance of a favourable water balance in the foliage. The exact role which the potash plays is not known. The maintenance of a favourable water balance in the foliage demands that loss of water by transpiration from the leaves shall not, except for short periods, exceed the supply of water to them through the shoot. It seemed desirable, therefore, to investigate the effect of potash supply on (1) the resistance offered to the flow of water to the leafy shoots, and (2) the transpiring surface, and to determine whether the ratio resistance/transpiring surface is affected by the level of potash supply.

Measurements of the resistance offered by shoots to the flow of water have therefore been made and a rough approximation to the transpiring surface of the leafy shoots has been obtained. An accurate determination of transpiring surface is not possible, but a first approach to the problem seems to be a determination of leaf areas supported by data for stomatal frequency. This has been done and it has been shown from what follows that the ability of cut shoots to transmit water when compared with the area of leaves to be supplied, is lower in material from trees with a deficient potash supply than in material from trees in which the supply of potash is adequate.

EXPERIMENTAL.

1. Material.

Material from two sources was used in the investigation.

(a) *Long Ashton.* Shoots were obtained from two sets of trees receiving different manurial treatment. The trees were of the variety Lane's Prince Albert, on Malling No. II rootstock. They were planted as maidens in December 1926, and since that date have been under a system of arable cultivation.

Manures have been applied each year as under:—

	Complete fertilizer plot. cwts./acre.	*No Potash plot.* cwts./acre.
Nitrate of Soda	2	2
Superphosphate	3	3
Sulphate of Potash	3	—

These will be referred to as " potash " and " no potash " material respectively.

There was here a large and obvious response to the potash, the no potash trees being much smaller than those receiving potash and exhibiting typical symptoms of potash starvation.

(b) *Goostrey* (*Cheshire*). Shoots here were obtained from two sets of trees of the variety Bramley's Seedling, on Crab rootstock, planted as 3-year-old trees in the winter 1919-20. One plot has received no manure and the other 3 cwt. of sulphate of potash per acre each year for the last six years. No nitrogen or phosphate has been supplied. As the trees are under a system of arable cultivation, nitrogen supplies are likely to have been adequate. No signs of phosphate deficiency are evident and it is extremely unlikely that any such deficiency exists.

2. Methods.

Comparable leader shoots were taken during July and August from two sets of trees, cutting at the base of the previous year's growth. Only shoots not under the direct influence of fruit were used. The shoots were removed to the laboratory at once and the following determinations made:—

(1) *Length of growth in current year.* This gives an approximate measure of the response to potash.

(2) *Number of leaves per shoot.*

(3) *Leaf area per shoot.* All the leaves on the selected shoot were removed and tracings made of them on paper of good quality. The leaf tracings were cut out and weighed and the total area of foliage carried by each shoot was calculated.

(4) *Stomatal frequency.* It was not practicable to peel off the lower epidermis to make stomatal counts. It proved more convenient to scrape the lower surface of the leaf with a sharp scalpel, and to mount the scrapings for examination. For each leaf about thirty microscope fields were counted.

(5) *Conductivity of shoots.* For these determinations a portion of the distal end of the previous year's shoot, about 15 cm. in length, was

selected, so that it was possible to measure the resistance to the flow of water to the shoot of the current year's growth. Pieces of stem 15 cm. in length were cut and placed in recently boiled and cooled distilled water and the vessel of water with the pieces of shoot in it kept under reduced pressure until no further air bubbles escaped from the shoots. Release of the diminished pressure caused injection of the shoots by the water.

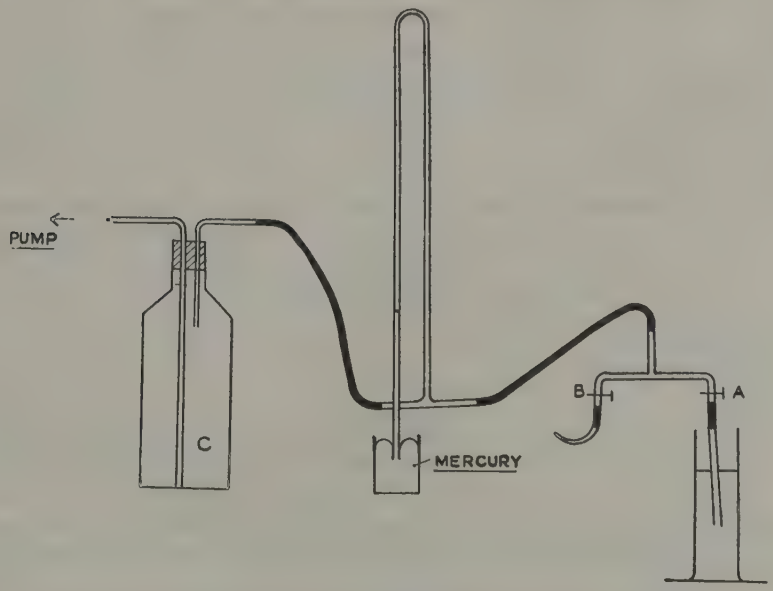

FIG. 1. Apparatus used for determining Water Conductivity of Shoots. For explanation see text.

Measurements of the ability of these pieces of shoots to transmit water were made with the apparatus illustrated. The tap A was closed, and by suitable adjustment of tap B connected to a capillary, constancy of pressure was attainable. A pressure of about 30 cm. of mercury was employed. During an experiment, provided a fairly large reservoir of air (C) was in circuit, variations in pressure usually amounted to only 2-5 mm. (i.e. possible error of about 1·5%). When greater variations than this were obtained the experiment was discarded. The shoots were fixed firmly to the tube connected with tap A, with the lower end of the shoot immersed in a weighed cylinder of water. Tap A was then opened and suction applied for about ten minutes, tap B remaining open meanwhile. The cylinder was then re-weighed and the difference between

this and the previous weight gave the amount of water that had passed through the shoot. From these data a figure called the "actual conductivity" was calculated. This is the amount of water which would pass through a 15 cm. length of stem in 15 mins. with a pressure of 30 cm. of mercury. These units, of length, time and pressure, were chosen as Farmer (2) employed them in his determinations of specific conductivities of the woods of evergreen and deciduous trees. It was assumed for the purpose of calculation that the water transmitted is directly proportional to time and pressure.

RESULTS.

The data obtained are incorporated in Table I. Mean values are given, together with the standard errors of the mean.

TABLE I.

	Long Ashton Material.		Goostrey Material.	
	No Potash.	Potash. (Mean Values ±	No Potash. Standard errors).	Potash.
Number of Shoots	21	14	11	12
Length of Shoot cm.	12·9 ± (1·51)	36·9 ± (2·26)	16·7 ± (1·77)	24·1 ± (1·21)
No. of leaves per shoot	11·9 ± (0·92)	19·4 ± (0·95)	14·6 ± (0·83)	16·6 ± (0·47)
Leaf area per shoot cm.^{2}	218·4 ±(16·9)	419·6 ±(29·8)	466·6 ±(24·0)	548·5 ±(34·0)
Stomatal frequency per mm.^{2} ..	478·8 ±(13·2)	382·3 ±(10·7)	329·2 ± (9·82)	282·0 ± (8·64)
Actual conductivity gm. H_2O ..	1·94 ± (0·366)	5·36 ± (0·311)	6·74 ± (0·61)	8·33 ± (0·53)

From these data certain other values have been calculated and these are tabulated below.

TABLE II.

	Long Ashton.		Goostrey.	
	No Potash.	Potash.	No Potash.	Potash.
Mean area of leaf (cm.^{2})	18·35	21·58	31·91	33·04
Number of stomata per leaf, in 1,000's ..	871	825	1,050	932
Number of stomata per shoot in 1,000's ..	10,370	16,041	15,360	15,468
Conductivity, gm. per 100 cm.^{2} leaf	0·83 ± (0·107)	1·32 ±(0·077)	1·47 ±(0·136)	1·53 ±(0·170)
Conductivity, gm. per 10^6 stomata	0·175	0·345	0·446	0·543

DISCUSSION.

Reference to Table I shows that for the Long Ashton material the values obtained in each case for potash and no potash material differ significantly. The response to potash is reflected in the increased shoot growth, the length of the current year's shoots being increased by almost 200%, whilst the total leaf area per shoot is almost doubled. Stomatal frequency is significantly lower in the potash material. The conductivity of the shoots differs markedly. The larger shoots of the potash material transmit a much greater amount of water than the no potash shoots.

The results for the material from Goostrey are similar, but the differences here are much smaller and often only just reach or nearly reach the level of significance.

Turning to Table II, it is seen that potash has increased the mean leaf size, and with this is to be correlated the decreased stomatal frequency. For Seakale beet (6) it has previously been shown that increased potash supply results in the development of larger leaves, the increase in leaf size being effected through an increase in cell size and accompanied by a decrease in stomatal frequency. The number of stomata per leaf is unaltered. A similar result has been obtained here. It is possible here to obtain a calculated value for the total number of stomata per leaf, and slightly fewer stomata are found in the larger leaf of the potash material than in the smaller leaf from the no potash shoots. Having regard to the method of calculating this figure, no significance can be attached to the difference.

The calculated statistics referring to the relation between the water transmitted to the shoot and the leaf area indicate a relatively deficient supply of water to the foliage of the no potash material. If the basis of expression instead of being leaf area is number of stomata, the no potash material occupies a still more unfavourable position. Under the stated conditions of length, time and pressure, in the no potash material, 0·175 gm. of water is transmitted per million stomata as compared with 0·345 gm. for the potash material. This refers to the material for Long Ashton. For the Goostrey trees, where potash deficiency is less severe, the difference is smaller but in the same direction.

The increased stomatal frequency in the no potash material can probably be correlated with the higher transpiration rates found by Mann (3) for the foliage of potash-deficient apple trees under conditions of abundant water supply and high light intensity. If the water supply is near deficiency point, it is to be expected that the leaves of the no potash material will feel the effects of the deficiency before the foliage of plants receiving a full potash supply, due in part to the greater stomatal frequency. Owing to the lower transmitting power of the shoots of the no potash material, a deficient supply to the leaves may

occur earlier than with the potash material. When the supply of water to the roots is abundant this may be of little importance. With a full water supply the relatively inefficient shoot of the no potash trees may be able to transmit sufficient water for the requirements of the foliage. With a lowered water supply to the roots, it seems likely that only the efficient shoots of the potash material can maintain a favourable water balance in the leaves. Hence it is to be expected that potash deficient trees will show signs of water shortage earlier than trees with a full potash supply, which is in accordance with field observations. The data presented, too, accord with observations of Wallace (5) that apple trees receiving a small supply of potash show much less evidence of potash starvation when the water supply is maintained at a high level. Abundant water mitigates certain effects of potash starvation.

The relative inefficiency of the shoots of the no potash material might be of less importance if a greater force were available for overcoming the resistance to the flow of water. Such data as are available, however, indicate that the osmotic pressure of sap expressed from the tissues (and hence probably the maximum suction pressure of the cells) is lowered when the potash supply is reduced (6).

It is concluded, therefore, that one effect of a deficient supply of potash to apple trees is to reduce the conductivity of the shoots. The conductivity is reduced to a greater extent than leaf area is reduced, so that the ratio conductivity of shoot/leaf area to be supplied, is lowered when the supply of potash is deficient. Owing to the increased stomatal frequency, a still greater reduction occurs in the ratio conductivity of shoot/number of stomata to be supplied.

This, it is suggested, is one of the reasons why potash-deficient apple trees are extremely susceptible to the effects of water shortage.

SUMMARY.

1. Observations have been made on the conductivity (of shoots), leaf area and stomatal frequency, of shoots of apple trees receiving a full supply of potash and of trees whose supply of potash is deficient.

2. Material from two sources was used in the investigation.

3. It is shown that the effect of potash deficiency is to

(a) significantly decrease shoot length ;
(b) ,, ,, number of leaves per shoot ;
(c) ,, ,, area of foliage carried by the shoot ;
(d) ,, ,, the ability of the shoot to transmit water ;
(e) ,, increase stomatal frequency ;
(f) ,, decrease the ratio conductivity/leaf area ;
(g) decrease (probably significantly) the ratio conductivity/number of stomata.

4. It is suggested that the reduction in the ratios conductivity/leaf area and conductivity/number of stomata is one of the reasons why potash deficient trees are so susceptible to drought conditions.

ACKNOWLEDGMENTS.

The Author's best thanks are due to Professor B. T. P. Barker for permission to carry out part of this work at the Long Ashton Research Station. Assistance in obtaining suitable material was readily given by Dr. R. Stewart and Dr. T. Wallace and is gratefully acknowledged.

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INVESTIGATIONS ON EGG-KILLING WASHES

II. THE OVICIDAL PROPERTIES OF HYDROCARBON OILS ON *APHIS POMI* DE GEER

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THE experiments of Tutin (9) on the effect of tar oils on the eggs of the Winter Moth, *Cheimatobia brumata* L., lead to the hypothesis, put forward by Staniland, Tutin and Walton (7), that ovicidal action is due to the physical stifling action of the oil deposit. Provided that the chemical stability of tar oils is kept constant, ovicidal efficiency should increase, up to a point, with increase in the boiling ranges of the oils. This conclusion has been verified in so far as tar oils are concerned by the further field work of the Long Ashton investigators on the control of the capsid bugs *Lygus pabulinus* L. and *Plesiocoris rugicollis* Fall. and by the latter work of Tomaszewski and Fischer (8) on the eggs of the silkworm, Winter Moth (*C. brumata*) and Apple Sucker (*Psyllia mali* Schm.). Further, the hypothesis has been confirmed experimentally for hydrocarbon oils of mineral origin, in particular by Austin, Jary and Martin (1), using eggs of *L. pabulinus* as test material. The evidence assembled by Martin (5) is unanimous in indicating that the insecticidal properties, in general, of petroleum oils increases with the boiling range of the oil until maximum efficiency is reached. The characteristics of a hydrocarbon oil suitable for the control of Capsid Bug or Winter Moth at the egg stage may, therefore, be regarded as known.

Petroleum oils of the usual types fail, however, to inhibit the hatching of aphis and psyllid eggs. Staniland, Tutin and Walton, therefore, suggested that ovicidal action on such eggs is associated with a chemical toxicity possessed by tar oils but not, in general, by petroleum oils. From this hypothesis it does not follow axiomatically that chemical stability and non-volatility are important factors in determining the toxicity of tar oils to aphis and psyllid eggs. Tutin stated, however, that in preliminary experiments with eggs of *Aphis pomi* De Geer, high-boiling tar oils were found more toxic than low-boiling oils, though he did not quote experimental results in support of this statement. Hartzell and Parrott (3), from critical studies on eggs of a number of insect species, were unable to substantiate Tutin's statement, for, although certain of their results were explainable upon this hypothesis, their tests on eggs of *A. pomi* indicated that relatively low-boiling tar oils are highly toxic. For this reason and because of the low boiling ranges of certain commercial preparations used

with apparent success on the Continent, Martin (5) included in the standard specifications which he suggested for consideration by manufacturers a Grade B oil of relatively low boiling range for use when only chemical toxicity to aphis and psyllid eggs was required of the oil. Because of this discordance, the primary purpose of the work to be described below was the examination of the comparative efficiencies of high and low boiling oils as ovicides against *A. pomi*.

A second object of the work has been the application of the dimethyl sulphate solubility test to the differentiation of those hydrocarbon oils most effective against aphis and psyllid eggs. The broad distinction that petroleum oils are ineffective whereas tar oils are effective as toxic ovicides lacks precision, and an analytical method is required which will evaluate toxic properties. Petroleum and neutral tar oils are both hydrocarbon oils differing in that the former are relatively poor whereas the latter are rich in aromatic hydrocarbons. Austin, Jary and Martin (1) accordingly used the Valenta test for the separation of aromatic hydrocarbons by virtue of their solubility in dimethyl sulphate. They found that this test successfully indicated those of a number of oils which were effective in the field in the control of *Psyllia mali*. It was considered necessary to substantiate this observation by laboratory tests upon aphis eggs.

Since the start of this work, Tomaszewski and Fischer (8) have published the results of their trials in which, among other factors, the influences of boiling range of tar oils and their solubility in dimethyl sulphate on the toxicity to eggs of *P. mali* were investigated. Unfortunately these authors gave no data by which the probable error of their results can be deduced and, although their results undoubtedly point to the greater toxicity of oils of high boiling range, it is impossible to trace any marked influence of the dimethyl sulphate solubility figure. It would seem, from their results with a given oil at different concentrations, that the field method they used demonstrated only large differences in toxicity.

Finally, an attempt has been made to determine the particular compound or types of compound to which the toxicity of tar oils to eggs of *A. pomi* is due.

EXPERIMENTAL METHODS.

Permanent Apple Aphis (*A. pomi*), was used as the test insect, as this species is the only one of the fruit aphides that can be reared successfully to lay eggs in large numbers under controlled conditions. Further, this species is more readily handled in the laboratory than others ; in particular, the mode of egg laying permits of the easy removal of the eggs from bark for the purpose of counting. The eggs of other species, such as *Anuraphis roseus*, are much more sparsely distributed and it would necessitate the treatment and examination of a very considerable number of shoots in order to obtain the number of eggs

required for each test. Field experience has shown that the eggs of the various fruit tree aphides exhibit susceptibilities of the same order to tar oil washes.

Shoots bearing eggs were cut during dry weather in winter and taken to the laboratory. They were carefully selected so that each had several hundred eggs of normal and uniform appearance. Not less than four shoots were used for each individual test and they were dipped into the insecticide, care being taken to agitate the fluid so that any air film on the shoots or around the eggs was broken. The shoots remained in the fluid for 15 seconds. After treatment they were attached to a strip of wood so arranged that the formation of areas of high concentration of the wash during drying was avoided. The basal ends of the dried shoots were cut off and rejected and the cut ends thrust into damp sand in pots. The pots were allowed to remain out of doors, exposed to the weather, until the first few eggs on the untreated controls showed signs of hatching, which usually occurred 4-10 days after the outer egg membrane had split longitudinally. The shoots were then taken into the laboratory and eight samples of each treatment (lengths of $1\frac{1}{2}$-$2\frac{1}{2}$ in.) were cut at random. Each of these lengths was impaled on a pin held by a cork inserted into an open-ended tube of $5 \times \frac{5}{8}$ inch dimensions. The open end was afterwards plugged with cotton wool. During the above sampling the twigs were handled in such a manner that none of the eggs was damaged. The tubes were arranged in trays to facilitate inspection.

In most instances the hatched nymphs wandered away from the pieces of shoot and in due course died in the tube. The cotton wool plug permitted the shoot and aphides to dry out without decomposition or development of moulds.

The total number of eggs on each shoot was counted after removal of the eggs by gently heating the shoot in 10% caustic soda in a water bath for about 10 minutes. Care was taken to avoid decomposition of the bark by excess heating. The eggs were filtered off, washed with weak acid and counted in 5-inch Petri dishes over squared paper. The aphides were readily counted by shaking and brushing them into a Petri dish. On each piece of shoot there were not less than 100 eggs and in most cases there were 300.

In the 1935 trials, the stock of aphis eggs was obtained from the new wood on an apple stool bed. The bed was carefully sprayed in August to remove all species of Hemiptera. The shoots were then infested with colonies of Permanent Apple Aphis, which bred very rapidly on the vigorous new growth and laid numerous eggs during October.

In the 1936 trials, the stool bed material failed, owing to unfavourable weather conditions, and material was therefore collected from a number of Worcester Pearmain nursery trees, considerable care being taken to collect uniform material.

In both seasons the treatments were carried out during the last week of January ; hatching began at the end of March and was completed by mid-April. The majority of the eggs hatched during the first week of April.

Parasitism of the eggs was not observed during the two seasons' experiments. Observations were made on the influence of the condition of the shoot on the viability of the eggs. It was found that eggs laid on weak shoots and stools, or on shoots covered with apple Scab lesions were frequently of low viability. In the experiments care was taken to eliminate shoots of this type.

MATERIALS USED.

Biological laboratory tests of the materials were carried out during two seasons, 1935-6. The oils and preparations are designated in the tables by letter and number, the tar oils are identified by the letter T, the petroleum oils by P, and the commercial preparations by C.

Representative samples of Grade A tar oils were employed together with oils of lower boiling range, certain of which conformed to the requirements of Grade B specification. The results obtained when these oils were subjected to analysis by the methods described by Martin (5) are recorded in Table I. The oils T.10 and T.11 are both Grade A oils ; T.12 and T.13 come within the limits proposed for Grade B oils except that T.12 contains a small percentage of neutral oils, insoluble in dimethyl sulphate, whereas T.13 is of slightly low specific gravity. T.14, a low-boiling creosote oil, is perhaps non-typical in that the tar acid content is abnormally low. The oil T.13 was therefore fractionated into a high boiling fraction, T.13a, so selected that the oil remained liquid on cooling, and a low boiling fraction, T.13b. Although anthracenoid solids were deposited from the neutral oils of T.13a, no difficulty was met with during the preparation and application of the ovicide.

In connection with the work on the identification of the compounds responsible for the toxicity of tar oils to aphis eggs, the neutral oils of T.11 were treated for the removal of oxygenated derivatives. As the physical constants of the untreated neutral oil, which was given the code number T.11a, are those quoted for T.11, neither this nor the treated oil T.11b, appears in Table I.

In addition to the tar oils, a number of commercial preparations were included in the tests. C.1 and C.2 are samples of a winter wash preparation widely used by growers to whom it is described as a tar oil wash. This preparation is of the miscible oil type, whereas C.3 is a sample of a stock emulsion also widely used by growers. The latter produce is guaranteed by the makers to satisfy the requirements of the Grade A. Type S.E. specification. The sample C.4 is of a proprietary disinfectant submitted by the makers with particulars of composition, for an opinion of its merits as a fruit tree wash. On the analytical

evidence alone it was considered unsuitable for such a use, but the material was included in the tests because of the low boiling range of the neutral oils, its relatively high content of tar acids and its interest for comparison with T.14. The products C.5 and C.6 are samples of experimental stock emulsions compounded from hydrogenated tar oils. Hydrogenation has apparently effected a reduction of part of the aromatic derivatives to hydrocarbons insoluble in dimethyl sulphate. Analysis of these products gave no difficulty except that, with C.6, the fraction of neutral oil insoluble in dimethyl sulphate separated as a yellow solid which, on standing, ultimately formed a liquid layer the volume of which could be determined. A check determination was carried out with ethylene glycol monoacetate, a preferential solvent suggested by Edwards and Lacey (2) for the separation of high-boiling tar and petroleum oils ; 19 per cent. by volume of the neutral oils proved insoluble in this reagent. A further point of interest met in the analysis of these products was the evolution of hydrochloric acid gas during the initial stages of the determination of the boiling ranges of the neutral oils of both products. It would appear that, during the extraction of tar bases with dilute hydrochloric acid, oxonium compounds are formed which, though stable enough to withstand the removal of solvent, are decomposed at the higher temperatures required for distillation.

TABLE I.

Analysis of tar oils and preparations.

	% by weight.			Neutral Oils.					
					Boiling Range.				
	Neutral oil.	Tar acids.	Tar bases.	Sp. Gr. (60° F.)	10% by vol.	50% by vol.	80% by vol.	Unsulphonated residue : % by vol.	Dimethyl sulphate : % by vol. insoluble in
T.10	89·4	4·1	5·4	1·063	274	337	390	—	0
T.11	90·6	4·0	—	1·104	267	324	365	—	0
T.11a	100·0	0·0	0·0	—	—	—	—	—	—
T.11b	100·0	0·0	0·0	—	—	—	—	—	—
T.12	86·1	8·4	4·7	1·049	253	306	352	—	6
T.13	87·4	10·7	4·0	1·040	254	307	349	—	0
T.13a	92·4	8·6	5·4	1·066	298	333	372	—	7
T.13b	85·9	10·7	4·0	1·009	234	260	292	—	4
T.14	91·8	2·3	4·0	0·974	203	234	276	—	11·5
C.1	62·4	8·1	2·0	1·010	243	320	376	—	33
C.2	64·6	8·4	3·4	1·002	249	310	363	—	31·5
C.3	63·2	2·5	3·5	1·079	280	330	371	—	0
C.4	30·6	19·8	1·2	1·026	222	246	300	—	0
C.5	68·2	1·3	2·5	1·036	329	371	400	12	36
C.6	67·5	1·3	2·1	1·073	335	365	400	—	22

Table II.

Results of biological tests of tar oils and preparations.

Test Insect—*A. pomi.*

Year of Test		1935.		1936.	
Code letter, number and concentration of material.		No. of eggs in test.	Mean per cent. hatch.	No. of eggs in test.	Mean per cent. hatch.
	per cent.				
T.10	2·0	1,545	9·5 ± 4·52		
T.10	1·0	3,313	44·6 ± 4·49		
T.11	2·0	—	—	2,621	2·7 ± 0·87
T.11	1·5	—	—	2,990	10·7 ± 2·32
T.11a	2·0	—	—	1,686	3·8 ± 1·62
T.11a	1·5	—	—	1,976	6·8 ± 2·04
T.11b	1·5	—	—	3,316	5·4 ± 0·97
T.12	2·0	3,432	26·9 ± 3·76		
T.12	1·0	1,767	54·7 ± 6·25		
T.13	2·0	1,672	18·9 ± 4·18	2,234	80·2 ± 3·16
T.13	1·5			2,506	56·9 ± 4·12
T.13	1·0	1,088	34·2 ±12·74		
T.13a	2·0	1,630	1·0 ± 0·42		
T.13b	2·0	1,436	60·8 ± 3·85		
T.14	2·0	2,268	21·8 ± 4·79		
T.14	1·0	1,835	63·1 ± 8·28		
C.1	4·0	1,689	20·9 ± 4·99		
C.1	2·0	2,647	40·9 ± 8·26		
C.2	3·0	—	—	1,837	12·8 ± 2·88
C.3	3·0	—	—	1,546	2·24 ± 0·90
C.4	4·0	1,456	57·7 ± 7·45		
C.4	2·0	1,332	33·2 ± 7·94		
C.5	3·4	—	—	2,084	3·7 ± 2·00
C.6	3·0	—	—	1,842	0·0 ± 0·00
Soap + benzene	0·7 } 2·5 }	1,940	70·9 ± 4·89	1,934	79·5 ± 3·37
(1) Soap	0·7	1,876	54·3 ± 8·52		
(2) Soap	0·7	1,378	40·7 ± 5·17		
Untreated		1,423	53·8 ± 5·39	3,481	96·1 ± 1·49

For the test of oils rich in constituents insoluble in dimethyl sulphate, a number of petroleum oils and preparations were used, the analyses of which are given in Table III. P.19 and P.4b were samples of light lubricating oils, the former being a highly-refined oil, the latter being a semi-refined spindle oil. The preparation C.7, compounded from an oil similar in characteristics to P.4b, was of the stock emulsion type. The remaining two petroleum oils were residues from petroleum oil refinement, P.18 being a high-boiling sulphur dioxide soluble fraction isolated by the Edeleanu process. This oil contains aromatic hydrocarbons, for 23% of the neutral oils are soluble in dimethyl sulphate. The oil P.20, of which a complete chemical examination was not carried out, was included because it proved completely soluble in dimethyl sulphate, but the predominant constituents are sulphur derivatives and not hydrocarbons.

TABLE III.

Analysis of petroleum oils and preparations.

	% by weight.			Neutral Oils.					
					Boiling Range.				
	Neutral oils.	Alkali sol. (Tar acids).	Acid sol. (Tar bases).	Sp. Gr. (60° F.).	10% by vol.	50% by vol.	80% by vol.	Unsulphonated residue : % by vol.	Dimethyl sulphate : % by vol. insoluble in
P.4b	100	0·0	0·0	0·989	348	378	394	74	100
P.18	96·2	2·6	0·3	1·003	320	359	394	14	77
P.19	100	0·0	0·0	0·860	312	347	369	98	100
P.20	99·6	0·4	0·0	0·962	181	203	264	—	0
C.7	62·0	0·0	0·0	0·883	347	382	—	76	100

TABLE IV.

Results of biological tests of petroleum oils and preparations.

Test Insect—*A. pomi.*

Year of Test		1935.		1936.	
Code letter, number and concentration of material.		No. of eggs in test.	Mean per cent. hatch.	No. of eggs in test.	Mean per cent. hatch.
	per cent.				
P.4b	5·0			2,009	35·4 ± 8·92
P.18	8·75	1,064	16·9 ± 5·84		
P.18	6·5			1,688	26·6 ± 5·14
P.18	4·35	2,266	59·8 ± 5·16		
P.19	5·0	1,167	43·5 ± 7·18		
P.20	2·0	1,011	39·6 ± 9·16		
P.20	1·0	1,428	43·9 ± 2·83		
C.7	8·3	1,524	35·4 ± 5·53	2,334	31·9 ± 5·03
Soap + benzene	0·7 / 2·5	1,940	70·9 ± 4·89	1,934	79·5 ± 3·37
(1) Soap	0·7	1,876	54·2 ± 8·52		
(2) Soap	0·7	1,378	40·7 ± 5·17		
Untreated		1,423	53·8 ± 5·39	3,481	96·1 ± 1·49

For the preparation of the ovicides, the oils were emulsified by the two-solution oleic acid method (4), each wash containing, in addition to the required amount of oil, 0·6% oleic acid and 0·1% sodium hydroxide. When solid hydrocarbons were tested, the required weight was dissolved in benzene and to the solution the oleic acid was added, this mixture then being poured into the dilute sodium hydroxide solution completing the final volume of the wash. The amount of benzene used was sufficient to give a final concentration of

2·5% by volume. For check purposes, soap solutions prepared by the addition, with or without benzene, of oleic acid in dilute sodium hydroxide solutions were used and, in two cases, benzene was added in parallel trials to washes containing hydrocarbons. From the commercial preparations, washes were prepared by direct addition to distilled water in the proportions stated in Tables II and IV.

DISCUSSION OF RESULTS.

The results of the counts of hatched aphides and total eggs are expressed in Tables II and IV as the mean figure for the percentage hatch of each of the eight (or seven) samples of eggs, together with the standard error of the mean. For simplification of discussion, the results will be considered, as far as possible in connection with the particular point under investigation, namely :—

I. The Influence of Boiling Range on Ovicidal Properties.

To eliminate the chemical factors, it is necessary to consider only the results obtained with oils showing a high solubility in dimethyl sulphate (i.e. tar oils). These results have, therefore, been abstracted to form Table V in which, as an index of the boiling range of the oil, the temperature at which 50% distils over is quoted.

The results show that the ovicidal properties, to *A. pomi*, of the tar oils under consideration increase with boiling range, a conclusion in agreement with Tutin's statement. At concentrations of 2·0 and 1·5% oils conforming to the Grade A specification have given a lower percentage hatch than oils of lower

Table V.

The Influence of Boiling Range on Ovicidal Properties.

	Boiling range 50% distil below.	Mean percentage hatch.		
		2% oil (Total).	1·5% oil (Total).	1% oil (Total).
1935 :				
T.10	337° C.	9·5 ± 4·52	—	44·6 ± 4·49
T.13a	333° C.	1·0 ± 0·42	—	—
T.13	307° C.	18·9 ± 4·18	—	34·2 ± 12·74
T.12	306° C.	26·9 ± 3·76	—	54·7 ± 6·25
T.13b	260° C.	60·8 ± 3·85	—	—
T.14	234° C.	21·8 ± 4·79	—	63·1 ± 8·28
Untreated	—	—	53·8 ± 5·29	—
1936 :				
T.11	324° C.	2·7 ± 0·87	10·7 ± 2·32	—
T.13	307° C.	80·2 ± 3·16	56·9 ± 4·12	—
Untreated	—	—	96·1 ± 1·49	—

boiling range. It is of interest to note that at the highest concentration, these oils have not given complete control, an indication that the concentration of 2·5% neutral oil soluble in dimethyl sulphate (corresponding to approximately 3% Grade A oil), a figure arrived at from general experience in the field as the minimum content of a tar oil wash able to control aphis and sucker, is not excessively high.

II. The Influence of Solubility in Dimethyl Sulphate on Ovicidal Properties.

To eliminate, as far as possible, the effects of boiling range on ovicidal efficiency, the comparison, given in Table VI, is confined to high-boiling tar and petroleum oils. The temperature at which 50% by volume of the neutral oils distils over is therefore quoted in the table, together with the actual oil concentration used and the estimated content of neutral oils soluble in dimethyl sulphate. In each year's group, the various ovicides are arranged in order of decreasing content of neutral oils soluble in dimethyl sulphate. This figure was calculated on the assumption that the specific gravity of oils and preparations is 1·00, an assumption that introduces a slight but negligible error.

Table VI.

The Influence of Dimethyl sulphate Solubility on Ovicidal Properties.

	Boiling range 50% distil below.	% Neutral oils.		Mean percentage hatch.
		Total.	Sol. in dimethyl sulphate.	
1935:				
8·75% P.18	359° C.	8·4	1·9	16·9 ± 5·84
2·0 % T.10	337° C.	1·8	1·8	9·5 ± 4·52
2·0 % T.13a	333° C.	1·85	1·7	1·0 ± 0·42
4·0 % C.1	320° C.	2·5	1·7	20·9 ± 4·99
4·35% P.18	359° C.	4·2	0·95	59·8 ± 5·16
1·0 % T.10	337° C.	0·9	0·9	44·6 ± 4·49
2·0 % C.1	320° C.	1·25	0·85	40·9 ± 8·26
8·3 % C.7	382° C.	5·2	0·0	35·4 ± 5·53
5·0 % P.19	347° C.	5·0	0·0	43·5 ± 7·18
1936:				
2·0 % T.11a	324° C.	2·0	2·0	3·8 ± 1·62
3·0 % C.3	330° C.	1·9	1·9	2·24± 0·90
2·0 % T.11	324° C.	1·8	1·8	2·7 ± 0·87
3·0 % C.6	365° C.	2·0	1·6	0·0 ± 0·00
3·4 % C.5	371° C.	2·3	1·5	3·7 ± 2·00
1·5 % T.11a	324° C.	1·5	1·5	6·8 ± 2·04
6·5 % P.18	359° C.	6·25	1·43	26·6 ± 5·14
1·5 % T.11	324° C.	1·36	1·36	10·7 ± 2·32
3·0 % C.2	310° C.	1·94	1·33	12·8 ± 2·88
5·0 % P.4b	378° C.	5·0	0·00	35·4 ± 8·92
8·3 % C.7	382° C.	5·2	0·00	31·9 ± 5·03

It is evident from Table VI that the total concentration of hydrocarbon oils is no criterion of the ovicidal efficiency of the emulsions upon *A. pomi*, and that a high degree of correlation is shown between the ovicidal properties of the washes and their content of neutral oils soluble in dimethyl sulphate. This conclusion involves the assumption that the oils are of a sufficiently high boiling range, for it will be noted that the petroleum residue P.20 (Table IV), although completely soluble in dimethyl sulphate, was an inferior ovicide. At 2% the mean percentage hatch was 39·6 ± 9·16, a figure not significantly different from the hatch on untreated twigs. This failure is to be ascribed to the low boiling range of the oil.

These results afford further evidence that the division of hydrocarbon oils into tar oils and petroleum oils respectively is insufficient to differentiate oils suitable and unsuitable for use as aphis ovicides. The likelihood of the introduction of hydrogenated and low temperature tar oils, which may contain high percentages of oils of a non-aromatic character ; the use, on the Continent, of lignite tar oils which may likewise contain paraffinoid hydrocarbons ; the fact that certain types of high-boiling petroleum oils may contain appreciable amounts of aromatic hydrocarbons ; these three considerations make it necessary to adopt a more precise distinction. Unfortunately, although the term aromatic hydrocarbons goes far to meet requirements, there is no corresponding term to embrace the naphthenic, the unsaturated and the saturated hydrocarbons, which are the predominant constituents of hydrocarbon oils. The best alternative would seem to be the adoption of definitions based on the actual analytical process, even though this introduces the cumbersome expression " neutral oils soluble (or insoluble) in dimethyl sulphate ".

III. The Ovicidal Constituents of Neutral Oils Soluble in Dimethyl Sulphate.

The suggestion put forward in the above paragraph involves the assumptions that either the individual constituent hydrocarbons of high boiling neutral oils, soluble in dimethyl sulphate, possess similar ovicidal properties to aphis and psyllid eggs, or the relative proportions of these compounds are identical in any sample of these oils. These assumptions can be put to experimental test only with difficulty, for little is known of the actual compounds present in high-boiling hydrocarbon oils, and those compounds which have been isolated appear to be present only in small amount. Two methods of attack were used, the first being the chemical fractionation of the neutral oils and the second the testing of individual compounds. For the former method, the neutral oil was treated, in light petroleum solution, with hydroferrichloric acid (6), which removes certain of the oxygenated derivatives as a sludge. The percentage hatch obtained with this treated oil (T.11b) at 2% was 5·4 ± 0·97, which is not significantly different

from that of the untreated oil, 6·8 ± 2·04. It may therefore be concluded that the ovicidal constituents are not fractionated by the treatment.

For the trials of individual compounds, a number of hydrocarbons present in low- and high-boiling tar oils were used together with a few miscellaneous products. Diphenyl oxide, for example, was included because this compound proved the most toxic of the tar oil constituents tested by Staniland, Tutin and Walton (7) on eggs of *C. brumata*. Tetralin (tetrahydro-naphthalene) and dekalin (decahydronaphthalene) were tested because Woodman (10) suggested the use of these well-defined chemicals as ovicides. The mean percentage hatch of aphis eggs obtained from twigs treated with these various compounds are recorded in Table VII, from which it will be seen that the solid hydrocarbons, acenaphthene, anthracene, diphenyl, fluorene and phenanthrene, were used at 0·5%, whereas the liquid hydrocarbons, cymene, dekalin, mesitylene, pseudocumene and tetralin, were tested at 1%. These low concentrations were used because it was considered that, if one or more of these compounds is responsible for the ovicidal properties of tar oils, this toxicity should be shown at low concentrations.

TABLE VII.

Sprays (All contain 0·6% oleic acid and 0·1% NaOH).	1935. Mean percentage hatch.
Acenaphthene (0·5%) + benzene (2·5%)	53·6 ± 6·91
Anthracene (0·5%) + benzene (2·5%)	49·8 ± 4·11
Diphenyl (0·5%) + benzene (2·5%)	58·1 ± 8·90
Fluorene (0·5%) + benzene (2·5%)	60·8 ± 4·06
Phenanthrene (0·5%) + benzene (2·5%)	59·9 ± 3·60
Diphenyl oxide (dibenzofuran) (0·5%) + benzene (2·5%)	62·5 ± 3·72
Cymene (1·0%)	64·5 ± 5·35
Dekalin (1·0%)	53·6 ± 6·93
Mesitylene (1·0%)	58·0 ± 6·09
Pseudocumene (1·0%)	64·7 ± 4·94
Pseudocumene (1·0%) + benzene (2·5%)	68·8 ± 6·81
Tetralin (1·0%)	67·0 ± 4·39
Tetralin (1·0%) + benzene (2·5%)	46·2 ± 3·84
Benzene (2·5%)	70·9 ± 4·89

The results indicate that none of the compounds tested possesses, at the concentration used, any marked toxicity to eggs of *A. pomi*. The aromatic hydrocarbons which, on the score of solubility in dimethyl sulphate, might be expected to exhibit some degree of toxicity, apparently fail on account of their relatively low boiling point. The poor ovicidal action of diphenyl oxide to *A. pomi* may be contrasted with its high toxicity to eggs of *C. brumata*, though the concentrations (2·5% and 5·0%) used by Staniland, Tutin and Walton were greater than in the above experiments.

The conclusion to be derived from these trials is that there is no evidence of specificity of ovicidal properties among the fractions and products tested. From the results quoted in Table VI, however, it would seem that slight differences may exist in the ovicidal action of high-boiling neutral oils, soluble in dimethyl sulphate, from diverse sources. Thus, the figure 26·6 ± 5·14, obtained with 6·5% P.18 corresponding to 1·43% neutral oils soluble in dimethyl sulphate, is significantly inferior to that, 10·7 ± 2·32, obtained with 1·5% T.11, containing a lower (1·36%) amount of active oils. This difference cannot be explained on the basis of boiling range, for the 50% distillation point of the petroleum oil is 359° C., whilst that of the tar oil is 324° C. Further, the complete kill obtained with 3·0% C.6 (1·6% neutral oil soluble in dimethyl sulphate) suggests that hydrogenation has conferred an enhanced toxicity on the original aromatic hydrocarbons, a feature not revealed by the dimethyl sulphate test. On the other hand, if high-boiling tar oils exist which show ovicidal properties markedly inferior or superior to that represented by the dimethyl sulphate figure, it is probable that such oils would have been recognized in practical use. As no such cases have emerged it may be considered that the dimethyl sulphate test provides an adequate practical method of assessing the toxicity of high-boiling hydrocarbon oils to eggs of *A. pomi*.

SUMMARY.

1. A laboratory method for comparing ovicidal properties, employing eggs of *Aphis pomi* De Geer as test material, is described. By this method, it has been shown that :—

2. Ovicidal properties to *A. pomi* of hydrocarbon oils are determined by their content of high-boiling neutral oils soluble in dimethyl sulphate.

3. There is no evidence of large differences in the ovicidal properties of high-boiling neutral oils soluble in dimethyl sulphate derived from various sources.

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